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RESEARCH ON THE NEW CLOTTING FACTORS

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This report is concerned with the rapid development of blood coagulation, a specialized branch of hematology. Because of the progress made during the last fifteen years this decade and a half has been called the renaissance period of blood coagulation. The history of blood coagulation does not begin with this period. The large amount of valuable information contained in the older monographs and articles, published during the last century and even before, should not be overlooked since in many instances surprisingly accurate deductions were made on the basis of relatively simple and primitive experiments.

Generalized interest in problems of blood coagulation and hemorrhagic diseases began with the opening of this renaissance period. The development of practical methods for the experimental and clinical evaluation of prothrombin, *circa* 1935, gave the primary stimulus for further investigations. The next major advance was the identification of a series of new clotting factors during World War II and immediately thereafter. Investigations concerning these factors are still in progress and much has yet to be learned. During the last few years the introduction of prothrombin consumption or utilization tests has made possible better analysis of the quantitative interrelationships among clotting factors.

In this review of some of these recent developments, particular attention will be paid to their physiopathological significance.

The new clotting factors. The existence of an additional clotting factor, necessary for the conversion of prothrombin into thrombin, had been postulated by the Iowa group, Warner, Brinkhous, and Smith (1939), but since experimental evidence was not available, the methods for prothrombin determination were still presumed to be essentially specific for this substance.

Between 1941 and 1944 Donhoffer, Greiner, and Meskò (1941), Marx and Dyckerhoff (1943), and de Nicola (1944)

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had stated that in addition to prothrombin, at least one other factor was involved in prothrombin time, but such observations were contrary to the concepts which then predominated among workers in this field. However, in the same years and independently, data from other laboratories gave support to the concept that another factor was involved, and a little later the characterization of Ac-Globulin confirmed the existence of the new clotting factor. The terms, Ac-Globulin, Ware, Guest, and Seegers (1947); labile factor, Quick (1943); plasmatic co-factor of thromboplastin, Honorato (1947); globulin of Fantl and Nance, Fantl and Nance (1946); factor V and VI, Owren (1944 and 1947); proaccelerin and accelerin, Owren (1950); plasma prothrombin conversion factor (PPCF), Stefanini (1951); etc., are now considered to refer to a single protein clotting factor or its modified form in serum. Only in the last two or three years has general agreement been obtained on the identity of this factor.

Not all experimental data could be explained, however, by the presence in blood of such a factor. In 1947 and 1948 the possibility was suggested by Mann, Hurn, and Magath (1947); Owen and Bollman (1948); and MacMillan (1948) that another factor might be involved in thrombin formation. It appears that the first complete experiments on such a factor were performed in 1949. Thus, the observations concerning serum prothrombin conversion accelerator (SPCA), Alexander, DeVries, Goldstein, and Landwehr (1949); co-thromboplastin, Mann (1949); proconvertin-convertin, Owren (1950); prothrombin conversion factor (PCF), Jacox (1949) were not generally recognized as being related to the same substance until very recently. Some of the misunderstanding may have resulted from the way in which proconvertin-convertin was originally presented although later Owren (1951) rectified these discrepancies. In the earlier paper, proconvertin had been assigned the properties of prothrombin and prothrombin of proconvertin. The name, factor VII, has been suggested for this factor by Koller, Loeliger, and Duckert (1951). This term is noncommital, and this seems to be an important consideration since the mechanism of action is uncertain. By using the name, factor VII, there is an additional advantage—it does not

necessarily imply the existence of a precursor, a controversial point at the present time.

Recently de Nicola (1951) was able to establish the relative equivalence of the various reported factors to factor VII, and, at the Macy Conference on Blood Clotting and Allied Problems (1952), such a correlation was recognized. The terms, SPCA and its precursor, proconvertin-convertin, co-thromboplastin, factor VII, probably PCF and the prothrombinogen of Quick (1949), can thus be considered as equivalent. The earlier observations reported by de Nicola (1944) probably refer to the same factor.

Several studies on the physiopathology of the new clotting factors have been reported. The investigations of congenital deficiencies, described in detail by Baserga and de Nicola (1949), (1952), are most interesting since these studies have been concerned primarily with an isolated defect. The congenital deficiency in factor V or Ac-Globulin was first called parahemophilia, and, after the introduction of the term proaccelerin, hypoproaccelerinemia. For proconvertin or SPCA deficiency, Owren (1952) suggested the term hypoproconvertinemia. Among the acquired defects, several conditions have been characterized which involve a combined deficiency of two or three factors. The recent investigations relating to factor VII are of particular interest and, therefore, will be discussed in greater detail.

The "quantitative" determination of factor VII. The availability of a simple and reliable technic for the quantitative measurement of factor VII has been of great assistance in the study of the physiological and pathological variations in this clotting component, but quantitation alone has not been sufficient to determine its interrelationships with Ac-Globulin (factor V and VI). Nevertheless, there can be no doubt that quantitation is essential for the development of our understanding of the interrelationships among the clotting factors. The assay procedure for factor VII is based on the same principle as the one-stage method for prothrombin time. When originally described, the one-stage technic was believed to measure only the variation in prothrombin since all other factors were assumed to be constant. However, we now know that other fac-

tors are present in the system and that these factors may vary, thus affecting the prothrombin time. Therefore, a modification of the one-stage method, based upon the effect of the new clotting factors, has been suggested. If not only calcium and thromboplastin, but also prothrombin and Ac-Globulin are kept constant, it is possible to measure the variation in factor VII. Conversely, if factor VII, Ac-Globulin, calcium, and thromboplastin are kept constant, the variations in prothrombin will be detected. A similar procedure may be applied to the assay of Ac-Globulin (factor V).

These assay procedures are made possible because factor VII can be quantitatively removed from plasma by Seitz filtration. When bovine plasma is filtered twice, first through a 20 per cent asbestos filter and then through a 30 per cent asbestos filter, factor VII is quantitatively removed while 10 to 15 per cent of the prothrombin remains. Such a Seitz-filtered plasma can be used as a substrate for the "quantitative" determination of factor VII, since Ac-Globulin and fibrinogen remain in optimal amounts. If one wishes to determine the combined activity of prothrombin and factor VII, it is necessary to remove both factor VII and prothrombin from the substrate plasma. This can be accomplished by one filtration through a 50 per cent asbestos filter. By these methods, substrate plasmas can be prepared for the determination of factor VII and factor VII + prothrombin.

The "quantitative" determination of factor VII is carried out as follows: oxalated bovine plasma prepared by mixing 1 part sodium oxalate (1.34 per cent) and 9 parts blood and centrifuging, is kept overnight in a refrigerator at about 4° C. and again centrifuged to remove precipitates which form at low temperature on standing. The clear plasma is then filtered through an asbestos filter containing 20 per cent asbestos. The filtration can be done by aspiration or pressure, but excessive foam production must be avoided. It is possible to filter about 100 ml. through a 6 cm. diameter filter. The first few cubic milliliters of the filtrate must be discarded. The filtrate is then filtered a second time through a filter containing 30 per cent asbestos. A 20 per cent asbestos filter has been successfully substituted for the 30 per cent asbestos filter during the second filtration in recent experiments. The final filtrate is then distributed in small

test tubes and stored at about −20° C. For use in assay, a tube is thawed at 37° C. in a few minutes and used within the next 30–60 minutes.

The plasma to be tested is obtained in the same manner as for the prothrombin determinations: 1 part sodium oxalate, 1.34 per cent, and 9 parts blood are centrifuged to remove the cells. One part of plasma is diluted with nine parts of veronal buffer (pH 7.2–7.4) according to Michaelis. The determinations are carried out in a water bath at 37° C. by introducing, in order, 0.1 ml. thromboplastin (same preparation as for prothrombin time), 0.1 ml. filtered bovine plasma, and 0.1 ml. diluted plasma. The stop watch is started with the addition of the diluted plasma. After exactly twenty seconds 0.1 ml. calcium chloride, M/40, is added to the mixture in the test tube. The clotting time is the time between the introduction of calcium chloride and the formation of a solid clot in the test tube. An end-point can also be recorded by tilting the test tube in the water bath or by introducing a wire in the test tube and observing the appearance of the first fibrin threads. With this technic and our thromboplastin preparations, the clotting time for normal human subjects is 24–26 seconds. The factor VII activity in per cent of the total can be obtained by additional plasma dilutions and plotting the values obtained on double logarithmic paper (Fig. I). Plasmas from normal human subjects diluted 1:10 with buffer are conventionally considered to give 100 per cent values. If the thromboplastin extract is varied, the standard curve is accordingly modified. In our experiments we used a commercial thromboplastin preparation kindly supplied by Hoffman-La Roche A. G., Basel, Switzerland. (Ro 1-4476/230).

The significance of factor VII in relation to the other new clotting factors. The use of this method in various experimental and clinical conditions makes it possible to evaluate the significance of factor VII in relation to the other blood clotting factors. Decrease in factor VII has been observed by Koller, Loeliger, and Duckert (1951) and de Nicola (1952) during treatment with dicumarol derivatives in addition to prothrombin deficiency and the non-significant variations in Ac-Globulin (Fig. II). It has been stated that the variations in factor VII

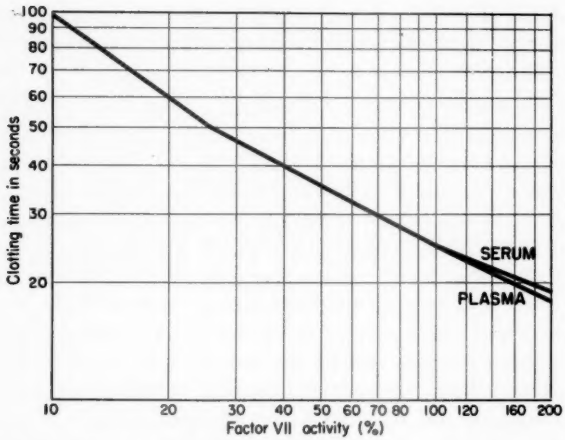

FIG. I. Standard curve, in double logarithmic system, for the "quantitative" evaluation of factor VII activity in percentages (100%=normal values in humans). Little or no difference is observed between plasma and serum for factor VII activities above 100%.

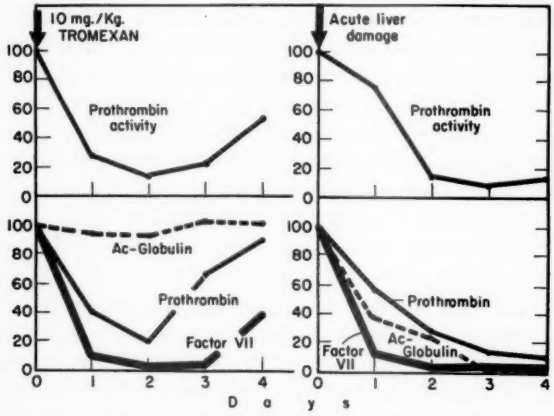

FIG. II. Left: Decrease in prothrombin activity (one-stage method) and in prothrombin concentration and factor VII activity during treatment with dicumarol derivatives; no significant variations in Ac-Globulin. Right: Decrease in prothrombin and factor VII activity and in prothrombin and Ac-Globulin concentrations in the course of acute liver damage.

during such anticoagulant treatment are more pronounced and earlier than the modifications of prothrombin. This lability of factor VII has been confirmed by the author. In the presence of liver injury, a decrease in factor VII, prothrombin, and Ac-Globulin are typically observed. Furthermore, a decrease in factor VII is an almost constant finding during the first days after birth (Fig. III). Thus, factor VII, prothrombin, and Ac-Globulin are all reduced in the newborn infant.

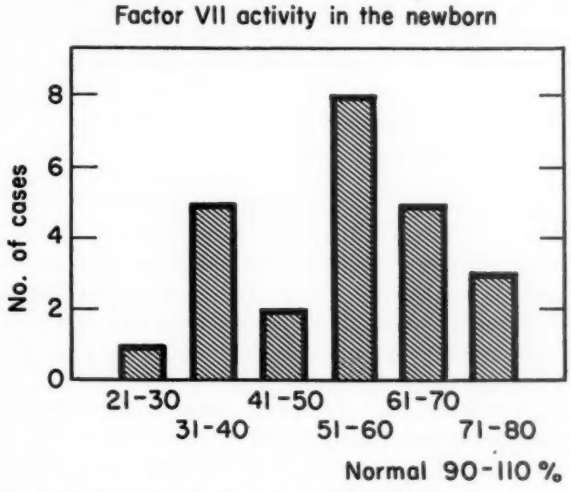

FIG. III. Decrease in factor VII activity in the newborn.

In contrast, increased factor VII activity has been observed by de Nicola (1952a) in thromboembolic diseases and by Koller, Loeliger, and Hu-Wang (1952) during pregnancy (Fig. IV). Other tests, such as prothrombin time, whole blood clotting time, etc., have usually given normal values in thromboembolic states, and therefore, if factor VII determinations detect an abnormality in a number of these cases, it may become a valuable test for clinical evaluation of a tendency toward intravascular hypercoagulability.

Data, demonstrating variations in factor VII under various conditions which are related to changes in the coagulability of

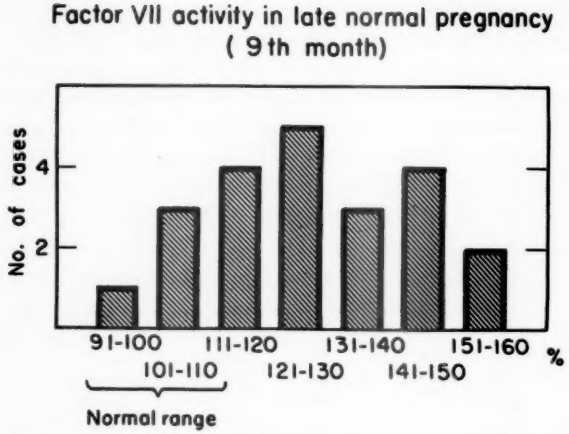

FIG. IV. Increase in factor VII activity during the late normal pregnancy (9th month).

the blood, indicate that this factor may be of considerable physiological significance. Experiments on clotting factors in various animals showed that the variations in factor VII are closely correlated with variations in prothrombin time, but not with variations in prothrombin concentration or Ac-Globulin (two-stage method). These results, as well as the observations in clinical conditions, seem to support the concept that factor VII is particularly concerned with the rate of prothrombin conversion and acts, therefore, as a real accelerator. Thus, prothrombin time appears to result from the composite effect of at least prothrombin, Ac-Globulin, and factor VII, but is especially influenced by variations in factor VII.

However, one must be cautious in interpreting these data to indicate a complete independence of factor VII from prothrombin. It is difficult to separate factor VII from prothrombin and, therefore, the two substances may have similar properties and behaviors.

The relationship of factor VII to the other clotting factors is not clear at the present time. Especially difficult to evaluate are the relationships between factor VII or allied factors and the thromboplastin factors. The discussion of this problem has

been intentionally postponed until a description could be given of the thromboplastin factors.

Thromboplastin factors; Quantitative interrelationships. Several investigators have attempted to characterize the phenomena concerned with thromboplastin activity which develop from blood components. It must be realized, however, that thromboplastin activity may also be derived from tissue sources, and therefore this tissue thromboplastin must be taken into consideration in physiological hemostasis. In blood, presumably uncontaminated with tissue juices, a thromboplastin effect can be obtained from the interaction of a platelet and a plasma factor. Quick (1947) has suggested that the plasma factor may be equivalent to antihemophilic globulin or to thromboplastinogen. Recently Shinowara (1951) and Johnson, Smathers, and Schneider (1952) have investigated this subject with purified reagents. The observations by Schulman and Smith (1952) and Aggeler, White, Glendening, Page, Leake, and Bates (1952), which relate other plasma thromboplastin factors to coagulation defects, will not be considered here as their position is not yet clarified.

The availability of relatively purified blood factors which are concerned with the formation of thromboplastin permits us to investigate, at least in part, the interrelationships among the various clotting factors. As a general rule, the alteration of one factor results in secondary alterations in other factors, and, for normal reactions to occur, the equilibrium state can not be greatly altered. A great advance in the interpretation of quantitative interrelationships has been the recognition that chemical processes are still occurring after a solid clot has been formed. When the amount of thromboplastin in the clotting system is reduced, the amount of prothrombin converted or utilized during coagulation also decreases. In hemophilia or in thrombocytopenia, the defect is characterized by a decrease in the plasma or platelet thromboplastin factor, and the whole clotting abnormality may be interpreted on this basis. In thrombocytopenia a deficiency in the platelet thromboplastin factor causes a secondary deficiency of thromboplastin. As Graham, Penick, and Brinkhous (1951) have indicated, even if the level of the plasmatic factor is normal, only a portion

of it then participates in the reaction. Consequently, an insufficient amount of thrombin is formed and, even in the presence of normal amounts of prothrombin, Ac-Globulin, and factor VII, only a fraction of each factor participates in the reaction. Soulier (1948); Baserga and de Nicola (1949); Quick, Shanberge, and Stefanini (1949); and Alexander, Goldstein, and Landwehr (1951), have demonstrated a decreased utilization of prothrombin and of Ac-Globulin or labile factor when such a situation prevails. Probably the same condition is extant in the presence of deficiencies of the plasmatic factor, *i.e.*, in hemophilia (Fig. V), but as yet we cannot determine the amount of non-utilized platelet factor. Shinowara (1951) has stated that a definite proportionality between the plasma and the platelet factor of thromboplastin appears to be necessary for optimal activity.

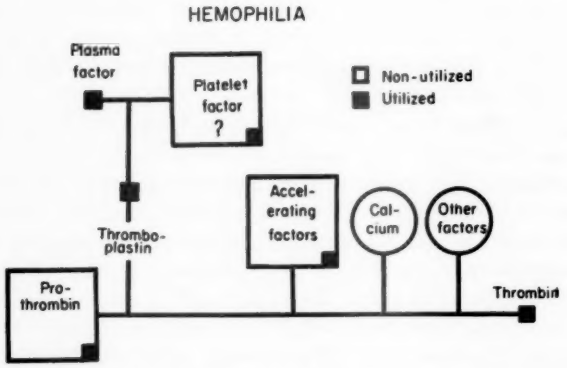

FIG. V. Illustrating the physiopathological consequence of a deficiency in the plasma factor of thromboplastin: secondary deficiency of thromboplastin and of thrombin, incomplete utilization of prothrombin and of accelerating factors, and (?) of the platelet factor of thromboplastin.

As with thromboplastin factors, definite proportions of prothrombin and calcium appear to be necessary for completeness of the clotting reaction. Thus, Quick (1947a) and Ware and Seegers (1948) demonstrated that, if the calcium concentration is above or below the optimum, the amount of prothrombin converted is decreased. In the study on factor VII and Ac-

Globulin by Koller, Loeliger, and Duckert (1951), it was concluded that reducing the amount of factor VII influenced the rate of the prothrombin conversion, but not the amount of the prothrombin converted. On the other hand, Ware, Guest, and Seegers (1947), have shown that reducing the amount of Ac-Globulin decreases the amount of prothrombin converted. The utilization or consumption of factors has been graphically represented in figure VI, in which prothrombin utilization and thrombin yield are reduced as prothrombin or Ac-Globulin are decreased. Thus, definite proportions should be considered in the interreaction between prothrombin and the so-called accelerating factors such as Ac-Globulin. Also, by reducing the amount of prothrombin, Alexander (1952) found a decreased utilization of Ac-Globulin or labile factor. The terms, utilization or consumption of factors, may not be completely correct because of limitations in the technics for precise quantitation. Nevertheless, consumption tests are useful clinical adjuncts

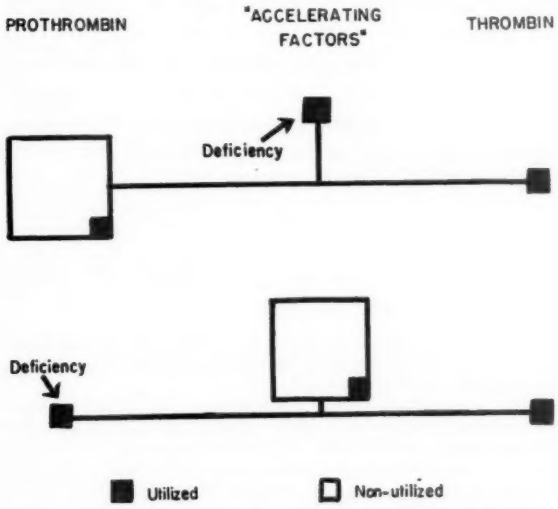

FIG. VI. Prothrombin utilization and thrombin yield are decreased in presence of decrease in either prothrombin or "accelerating factors" (Ac-Globulin).

and help in the interpretation of the fundamental coagulation reactions.

It has been suggested that the presence of active thromboplastin might be necessary for the activation of factors like proconvertin. If this is true, thromboplastin factors and proconvertin-convertin are interrelated in such a way that the deficiency in hemophilia and thrombocytopenia may be explained on a more rational basis. However, definite evidence of such a correlation is not yet available. Although several authors are convinced that the active form of the so-called converting factors derives from a precursor (SPCA and precursor, proconvertin-convertin), the experimental results of Koller, Loeliger, and Duckert (1951) do not agree with such a point of view.

From an analysis of the studies which have been briefly surveyed, it is evident that some details require further investigation: the characterization of the concepts "acceleration" and "conversion" and proper application; complete analysis of the quantitative interrelationships between the various factors; and the problem of activation of possible precursors of such factors as factor VII. Although previous investigations have not given the answer to such questions, they have indicated what the problems are. The search for the answers should be a stimulating future chapter in the history of our attempts to unravel the mysteries of blood coagulation.

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THE ARMED FORCES INSTUTUTE OF PATHOLOGY

ELBERT DECOURSEY*

The Armed Forces Institute of Pathology is a national institution administered by the Armed Services of the United States. It draws its strength from the medical profession at large through the channels of pathology, to serve the needs of civilian as well as military medicine. It was founded as the Army Medical Museum in the midst of one of the bloodiest wars of this nation, by The Surgeon General, W. A. Hammond, a man fully cognizant of the importance of research and training to the conquest of disease. From 1862 until 1920 major emphasis was on the Museum and its collection of morbid anatomical specimens. This provided an excellent supply of teaching material and it was natural that the Army Medical School should have been organized at the Museum and housed there during its early years. This venture coincided with the turn of the century, a vital period in our national medical history, and important contributions were made by men who worked simultaneously on both staffs.

In 1920 Major (now Brigadier General, retired) George R. Callender took the first step in leading the Museum to a more active participation in the study of living pathology. He met with a group from the American Academy of Ophthalmology and Otolaryngology to organize a Registry of Ophthalmic Pathology where material and records could be collected and cases followed up for the purpose of continuing research. Subsequently, the military and civilian pathologists, joined by other interested members of the medical, dental and veterinary professions, coordinated their efforts for the advancement of pathology through the formation of more such registries.

This action was to be of service to the civilian profession and of material assistance in the fulfillment of the first function of this institution—to serve the military all over the world. To this end pathology has been carefully organized in the military service since shortly after World War I. General Callender started the active participation of the Museum in pathology;

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Colonel James E. Ash, Curator from 1939 to 1946, made it a real institute of pathology; and General Raymond O. Dart, who followed him, organized it into the Armed Forces Institute of Pathology.

Today military pathology functions as a global enterprise on a plan that in graphic form is analogous to an ordinary target. A peripheral circle represents every ship, station hospital and small hospital dispensary in the military service anywhere in the world. Many of these units are too small to have a pathologist assigned to the staff. An inner concentric circle represents the system of general hospitals and histopathology centers throughout the world which meet this contingency. Each of these centers serves a segment of the larger circle of small units; thus, no matter where a service man may be stationed, his doctor has access to a good pathology laboratory. In these histopathology centers material from all autopsies, important surgical and biopsy specimens are screened before being forwarded for consultation to the AFIP, the central point, or bull's eye of the target. A small circle around this nucleus represents the civilian pathologists, consultants to the AFIP, to whom cases requiring extensive study, or of a controversial nature, are sent for review. Each consultant spends from one to four weeks a year in residence, being available for consultation and discussion of problems with staff pathologists, and lecturing on his specialty. Such an organization assures the best possible diagnostic services to the Armed Forces while concentrating in the AFIP a supply of material that is available not only to the military but to any member of the civilian profession for study, research and training.

From the early days of the Museum, the professional personnel have always worked in closest cooperation. Today a tightly knit staff composed of officers of the Army, Navy, Air Force, and Public Health Service, as well as physicians from the Veterans Administration and civilian life, functions within the framework of five interrelated departments. An Administrative Service supplies the needs of the other four departments; namely the Department of Pathology, the American Registry of Pathology, the Medical Illustration Service, and the parent organization—the Medical Museum.

The Department of Pathology contains approximately twenty special sections to cover various anatomic divisions and disease entities, such as Dental and Oral Pathology, Otolaryngic Pathology, Gastrointestinal Pathology, Neuropathology, Forensic Pathology, Radiation Pathology and the like. Each section is under the direction of a specialist, selected from the more than thirty pathologists now working at the institution. The organization of this department dovetails into the scheme of the American Registry of Pathology in that the head of a specialty section also serves as registrar of the corresponding registry.

The American Registry of Pathology, composed of twenty-two individual registries sponsored by national specialist societies, is partially civilian in character. It is under the auspices of the National Research Council, which handles the funds contributed by some of the national societies toward the support of their registries. While many of the societies do designate funds for this purpose, the greater part of the expense of this department is borne by the military. This is justifiable on the grounds that the Registry stimulates continuous contribution of pathologic material in special categories which cannot be supplied by the Armed Forces alone, yet is of inestimable value to the program of education and research.

When a registry is first established, the special field which it covers may be one of little interest to many pathologists. This was true of the first registry set up in the early twenties when few pathologists studied eyes. The registry program in the Armed Forces Institute of Pathology is believed to have been a factor not only in increasing the interest of clinicians and specialists in the pathology of their field, but also in encouraging them to foster a similar interest among pathologists. To achieve this end it has sometimes been necessary, early in the life of a registry, to accept from clinicians cases that have not been seen by a pathologist. Although this is not in accord with our policy, exception is made if pathologic service cannot be obtained locally. If on gross examination of such material a tumor or other dangerous condition is noted, for the benefit of the patient a microscopic study is made as soon as possible and the contributor is furnished with a report. It is emphasized that the American Registry of Pathology is not a diagnostic center and

this method of receiving material is abjured whenever possible.

Through the cooperation of a number of the established registries an outstanding contribution has been made to the furtherance of the education of the pathologists in the seminars of the American Society of Clinical Pathologists. The facilities of the AFIP have been utilized year after year to provide pathologic material and laboratory service for making the microscopic slides used in these seminars. For the 1952 seminar one thousand sets, each with twenty-five slides, were prepared.

The Ophthalmic Pathology Registry provides an excellent example of the value of the programs of the individual registries. The recent "Atlas and Textbook of Ophthalmic Pathology" is largely based on its material. A collection of twenty-five hundred melanomas gathered with the help of many doctors throughout the country and the world has provided the basis for a pathologic classification important in prognosis.

A new and exciting contribution from Helenor C. Wilder, the Registrar of the Registry of Ophthalmic Pathology, appeared in the August issue of the Archives of Ophthalmology. Her observations might have been made elsewhere, but they were not, and it is logical to believe that the accumulation of material in the Registry made her study more feasible. For years the diagnosis of tuberculous chorioretinitis has been made, but pathologists have rarely been able to demonstrate acid-fast bacilli, to transmit the infection to animals, or to identify an etiologic agent. Mrs. Wilder was able to demonstrate toxoplasma-like organisms in tissue sections from fifty cases of "tuberculous" chorioretinitis, that is, in twenty per cent of the total cases of granulomatous chorioretinitis in the Registry.

Another function of the Armed Forces Institute of Pathology in the field of education, or the stimulation of education, is of especial importance to military medicine. Recently our troops in Asia were stricken with a disease similar to leptospirosis. When it occurred in Manchuria just prior to World War II, the Japanese called it "epidemic hemorrhagic fever, cause unknown." More recently, when several hundred U.S. troops contracted the disease, it became a potential military danger. Military medical officers are naturally kept abreast of such urgent problems, but it is important that civilian pathologists also be

apprised of them in order to be of maximum assistance in providing a solution. To this end a special article on this little known disease was wanted for the Yearbook of Pathology, by its editor, Dr. Howard T. Karsner. The group working in Japan, who had been sending material to the AFIP, were asked to write an article. (It is not our practice to utilize contributed material for publication except with permission of the contributor. The individual working directly with the disease is encouraged to write the original article but to furnish the AFIP with material for further research). While the authors were enroute to this country, illustrative material was prepared and correlated with the paper so that it could be read before a national society which was meeting immediately after the authors landed. Thus, through the cooperation of the AFIP and its facilities this important information was prevenantly disseminated.

The Medical Illustration Service is also important to the educational program. Extensive files are maintained of the medical photographs and illustrations prepared by its artists. The many textbooks that contain illustrations obtained from this file attest to its usefulness. Prize after prize has been awarded to medical exhibits prepared for display at national meetings by the Medical Illustration Service.

The Medical Museum, in addition to a professional teaching program, has the difficult assignment of fostering a true understanding of medicine among the laity—an understanding not of its scientific content, but of its problems and objectives. Current interest in medical activities is clearly indicated by an increase of visitors to the Museum to well over 100,000 annually. High school and adult education groups are also using the Museum to supplement classroom programs. To amplify this mission, cautious but nevertheless decisive steps have been taken in the field of television. One of the networks has requested a thirteen-week sustaining program on which we are now working. As scientists it is our duty to accumulate knowledge and use it for the benefit of mankind. At the same time, we must keep the non-medical public informed, not so much of actual techniques and data or even of practical accomplishments, but rather of the evolution of scientific processes, exemplified in the introduction of new techniques and the develop-

ment of fresh concepts. The case history and the historical approaches to teaching are inherent in a museum and probably represent the best methods by which an understanding of medical science can be achieved.

Research, and the knowledge obtained therefrom, is in itself a potent weapon for peace. It is essential, therefore, that in this, as in all other fields, we keep not merely abreast but ahead of every other nation on earth. As long as a potential enemy realizes that no matter what he has, we shall have something better, his will to start a fight is undermined. To this end we have a mission to inform the public of the important advances in medical science and maintain support for this phase of the nation's defense.

A new AFIP is in process of building. It will consist not only of a new structure, but will contain the necessary additional scientific facilities for a well rounded program. In the past there has been little more than a division of morphology in the broad field of pathology. In the future there will be divisions of basic sciences and research pathology. The research approach will be charted by the requirements of pathology, but it will be supported by representatives of the related sciences: a bacteriologist, a virologist, chemists of varying interests, a statistician, a radiobiologist, a physicist, etc. These scientists will not work on projects related solely to these ancillary disciplines, but as members of a research team will direct their investigations toward the solution of the problems of pathology.

Naturally most of the activities of the Armed Forces Institute of Pathology are directed toward the advancement of military medicine, which now encompasses so many problems and concerns such a wide segment of our people. The material sent in by civilian pathologists and the consultative assistance given by them are important contributions to this mission. However, there is also the educational mission for military and civilian alike. Under the auspices of the Registry, loan study sets of microscopic pathology slides and lantern slides have been prepared, and the Illustration Service has on file motion pictures, prints and slides, all of which may be borrowed for your use as teacher or student. This material has been made easily available in appreciation of the support which is being given to the

AFIP by the civilian medical profession. Information regarding this material may be secured by writing The Director, Armed Forces Institute of Pathology, 7th and Independence Avenue, SW, Washington 25, D.C.

AN APPRECIATION OF WALTER B. CANNON

WALTER J. MEEK*

Before the personal charm of the man has become only a tradition and before the facts of his life have become only those recorded by the formal biographer, it is appropriate to review the life of Walter B. Cannon, one of the greatest of current physiologists. There are those who knew him far better than I, particularly Cecil Drinker long his colleague at Harvard, Hoskins, Rosenbleuth, and Binger. My own acquaintanceship and cordial friendship with Dr. Cannon were largely the result of our associations at the times of the annual meetings of the American Physiological Society. Dr. Cannon was an officer of the Society for a period of fifteen years. For five years of this time I served as a member of the Council either under him as President or with him as a fellow councilor. The Physiological Society in those days was a small compact organization and most of the members were at least well acquainted, if not close personal friends. One need not apologize that such a review as this may have some of the color of personal remembrances.

The important sources of biographical material for Dr. Cannon are the testimony of his friends, the records of the American Physiological Society, the notices by Drinker and Binger at the time of his death and his own autobiographical material in his book, *The Way of an Investigator*. In this really remarkable volume Dr. Cannon gives a good deal of his family history, the influences that affected his scientific career and illustrative discussions of the qualities which he considered valuable for a scientist.

Many of you may have visited the old village of Deerfield, Mass., to see the iron studded door which withstood the blows of Indian tomahawks in 1704. Forty-seven of the little village were massacred, their blood turning the little brook to crimson, and 112 were carried away as prisoners. Among the latter

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was Jacques de Noyon, a French Canadian coureur de bois who had married Abigail Stebbins, a daughter of one of the early settlers. Probably Abigail was supposed to have married beneath her station but her husband's acquaintance with French and the Indians was undoubtedly what saved father and mother Stebbins and their six children on the terrible road to Montreal. Jacques and Abigail finally settled on the south bank of the St. Lawrence not far from Montreal and the Stebbins family later made their way back to Deerfield. Gradually a whole flock of de Noyon children appeared and one of the boys, Reni, was allowed to go to Deerfield with some Indian traders to visit his grandfather. When the time for return came Reni could not be found. His grandfather does not seem to have been entirely innocent in this circumstance, for in due time the boy reappeared. His name had a rather heathenish sound and the religious grandfather renamed him Aaron. De Noyon became shortened into Denio, and so in a sense was born Aaron Denio whose descendants spread north to Vermont and then westward as far as Minnesota, Colorado, and Wyoming. From Minnesota one of the young women came down the Mississippi as far as Prairie du Chien where she had secured a position as schoolteacher. Her name was Sarah Wilma Denio and she later became the mother fo Dr. Cannon. Cannon.

On his father's side Dr. Cannon stemmed from an Irish immigrant to Boston in 1718, by the name of Samuel Carnahan, a blue blooded Presbyterian. The name rather quickly became Cannon and descendants of Samuel moved into the Western Reserve and eventually into Wisconsin, Minnesota, and Iowa. Dr. Cannon was proud that one of his great grandparents, Laura Cochran Cannon, walked beside a covered wagon all the way from Blandford, Massachusetts, to Ohio. Dr. Cannon's father was Colbert Hanchett Cannon who throughout his life was a railroad man eventually becoming a superintendent of transportation for the Great Northern. For a time he was stationed at Prairie du Chien. Here he met the young schoolteacher, Sarah Denio. They were married in 1870 and on October 19, 1871, their oldest child, Walter Bradford, was born.

In view of his future career in gastro-enterology it is an interesting coincidence that Dr. Cannon should have been born at Prairie du Chien where William Beaumont made his classic studies on digestion. However the family did not remain long at Prairie du Chien but moved to St. Paul where Walter Bradford's boyhood was spent. There is mention of a brief stay in Milwaukee but no details seem available.

Dr. Cannon describes his mother as having an active mind and being modest and unselfish. She died when the boy was only ten years old but a remark made on her deathbed showed a lofty sense of duty and influenced her son greatly throughout his life. It was "Walter, be good to the world." Dr. Cannon says these words fixed in his mind a "sacred and haunting memory." This influence of his mother explains, I am sure, many of the social and philosophical attitudes displayed in later years.

His father, too, greatly influenced his son's career, but in rather an unexpected way. Although his father wanted him to be a doctor there is no evidence that he guided Walter Bradford into this field. He did, however, force the boy to learn the use of tools. He would not buy ready-made toys, but he did turn over a fine carpenter's tool chest and encouraged the lad to make his own playthings. Dr. Cannon attributes his own skill and ingenuity for building apparatus as going back to these days.

Dr. Cannon's secondary school education was completed at St. Paul. He finished high school in three years, possibly because his father made him work in an office for two years when he was fourteen and this experience encouraged him to speed up his work. As a high-school student he was interested in all forms of sport and outdoor life. At the same time he showed a vigorous mind. He was editor of the high-school paper. He became greatly interested in Huxley and the liberal philosophers of his day. His pastor questioned his right to criticize the opinions of the great scholars who supported the church, and the result was that he withdrew from the church. This caused a rift between him and his father which was only completely healed in later years. Largely through the influence of his English teacher, a Miss Newson, he decided to go to

college and to Harvard. His resources were slender, consisting of a freshman scholarship and $180 from his father. Thereafter he was entirely independent supporting himself through four years of college and four years of medicine by the aid of scholarships and outside work.

No one ever heard Dr. Cannon complain about these years which might be termed by present students as years of penury. An example of the hardships he probably underwent came to my attention shortly after his death. At that time I wrote Dr. Cecil Drinker for some kind of a remembrance of Dr. Cannon to be placed in the historical rooms of the Beaumont Foundation at Prairie du Chien. Dr. Drinker sent me a long roll of brown wrapping paper on which were pasted side by side three long strips of toilet paper. On these latter were the X-ray outlines of the stomachs of three cats following a carbohydrate meal. Thin India or silk paper might have been nicer, but it was evident that the first-year medical student, for such he was, had no money for items not absolutely necessary.

He graduated from Harvard College in 1896 with a *summa cum laude* and at once entered the Harvard Medical School. At the end of his first year of medicine he was granted a Master's degree and at the end of the fourth year, 1900, the M.D. degree. His college life was influenced most largely by Charles B. Davenport, George H. Parker, and William James. All three of these famous scientists were biologists or at least biologically trained. Davenport, the eminent anatomist and geneticist, later head of the Cold Springs Harbor Laboratory, and Parker, the equally eminent physiological zoologist, both outlived their illustrious pupil. At one time Cannon confesses that in his eagerness to take all knowledge as his field, he thought of becoming a philosopher. James' advice however was, "Don't do it. You will be filling your belly with east wind." Cannon rather thought this was probably less an aspersion on philosophy than on his own ability. At any rate he took the advice and stayed with biology.

He had assisted as an undergraduate in teaching comparative anatomy and in his senior year of medicine he conducted this course at Radcliffe. This resulted in his being offered an instructorship at Harvard College in 1900 at the time he re-

ceived his M.D. degree. The Medical School however also made a bid for his services and he accepted an instructorship in physiology under Henry P. Bowditch, one of the most distinguished physiologists of his day and one of the three founders of the American Physiological Society. In 1902 Cannon was made an assistant professor and in 1906 he succeeded Bowditch as the George Higginson Professor of Physiology. In this capacity he served for thirty-six years until his retirement in 1942. He and Bowditch represent a period of seventy-one years which includes the entire developmental period of physiology as a professional branch of medicine and biological science in America.

During Dr. Cannon's undergraduate years a bit of romance had been developing or perhaps it would be more accurate to say flowering. Cornelia James, a young woman he had known in his St. Paul high-school days, was at Radcliffe. A year after Cannon received his degree from the Medical School, that is 1903, they were married. Cannon related that a colleague in his department at the time cynically remarked, "A young man married is a young man marred." Certainly anyone who has had the pleasure of knowing Mrs. Cannon would not agree, and Dr. Cannon always referred to his wife as his best "counselor and companion." In addition to being a wife and mother of five children, Mrs. Cannon has had a literary career of note, several first-rate novels having come from her pen.

Dr. and Mrs. Cannon were both great lovers of the outdoors. Their honeymoon was spent canoeing down the St. Croix and upper Mississippi. Following this they made for northwestern Montana and visited what is now Glacier National Park. At the upper end of Lake MacDonald they saw an impressive mountain mass known as Goat Mountain. With considerable difficulty and in spite of some danger they succeeded in climbing it. Several years later somewhat to their surprise Dr. Mathus, of the United States Geological Survey, sent them a topographic map of the region and "Goat Mountain" had become "Mount Cannon" which it still is or certainly was to my knowledge in the early '30s.

The Cannons, old folks and children, continued these out of door excursions for many years. Ford after Ford transported

the family to the Rocky Mountains and back again. In later years they spent more time in the White Mountains, having a summer home at Franklin, New Hampshire. One of my own most delightful memories was the honk of a Ford at our door one Sunday morning in summer and into the back yard trooped Dr. and Mrs. Cannon and two or three of the girls all in khaki and fresh from somewhere in the Rocky Mountains. The resourcefulness and patience of the explorer was part of Dr. Cannon's nature and he looked on these qualities as being among the prime requisites of a scientist.

Dr. Cannon's courtesy, good humor, accessibility and friendliness were personal characteristics familiar to all who came in contact with him. He liked people, young people in particular. He had a futhfulness of spirit which never left him even in the days of his infirmities. Two personal traits, or personal points of view, had a great deal to do with the activities of his mature scientific life. One was his natural inclination to espouse the cause of the under-dog; the other was his firm conviction of scientific internationalism and the close relation of science to a true democracy. In following these views to their natural ends he was often accused of ultraliberalism, of having lack of judgment and even of being a "Red." Although not exactly unperturbed by these criticisms he never ceased to labor for the Spanish Republicans, the Chinese Nationalists, and for better Soviet scientific understanding.

At the Fifteenth International Congress held in Moscow in 1935, in a fine speech at the opening session, he expressed his deep conviction on the high destiny of scientists and particularly on the vital role which physiologists play "for the benefit and happiness of mankind." He warned the Congress against the dangers which threaten fruitful scientific activity. These were the pernicious influences of political adventurers; failure to provide adequately for laboratories; freedom in selection of subjects for investigation; confinement to immediately useful projects or to a so-called nationalist program.

These remarks were directed toward a hateful fascism which soon involved the world in war. Some heard them with great enthusiasm, others with cynicism, but all realized that a great scientific idealist had spoken.

Dr. Negrin, professor of physiology and Dean of the Medical School at Madrid, who later became premier of the Republic, was a friend of Dr. Cannon. This encouraged Dr. Cannon to serve as national chairman of the Medical Bureau to Aid Spanish Democracy. I well remember an address he made in the Auditorium Theater in Chicago in favor of the Spanish cause. He talked freely to Dr. Carlson and myself about the propriety and value of his efforts, but his sympathy was stronger than any other feeling, particularly any fear of criticism. Although Dr. Cannon at that time was sometimes called a Bolshevik, no one who knew him could ever doubt his loyalty to his country. His sympathy for the Chinese at the time of the Japanese invasion was as great as it was for the Spanish and again he served with the American Bureau for Medical Aid to China and with the United China Relief. Dr. Cannon was in China, Manchuria, and Korea in 1935 when the Japanese entered these countries.

Another opportunity for usefulness in Dr. Cannon's opinion was to aid in a better understanding between the Soviet Union and the United States. At the various international congresses he had become a warm friend of Pavlov, Orbelli, and many other Russians. Although neither he nor Pavlov had much respect for Soviet theories it became apparent that the Soviet Union was destined to be a world power. Cannon believed that a good understanding between the scientists of the two countries was desirable, so when the American-Soviet Medical Society was organized he accepted its presidency. He frankly admitted that the venture was in the nature of an experiment. Later events have of course shown that the influence of a group of scientists has little effect on the entrenched policies of a Politik-Bureau.

Cannon, however, never ceased his fight for scientific liberation and it brought him the respect, confidence and affection of people throughout the scientific world. Only a few weeks before his death he joined Herbert Evans, John Fulton, and Carl Wiggers in a plea for funds to supply Professor B. A. Houssay with scientific journals. This was at the time Houssay was forced out of his laboratory by the fascist regime in the Argentine.

One result of Dr. Cannon's international relations and of course his exceptionable ability as a physiologist was a really tremendous influx of foreign students to the Harvard Laboratory. Every country in the world that had a physiological laboratory at some time or other sent students to study with him. Many of these have become eminent and all have carried the spirit of the Harvard laboratory to their homeland.

He was of course equally cordial to American workers, particularly the younger physiologists who wished to gain experience in his laboratory. My own plans were once made to spend a semester with him and although I never succeeded in carrying them out I remember his cordiality with the utmost pleasure.

Dr. Drinker said, "It is our habit to seek for influences which start men of great accomplishments upon their careers. Usually such efforts result in highly artificial syntheses inappropriate to the men involved." At the twenty-fifth anniversary dinner of his doctorate, Dr. Cannon described himself as the son of Dr. Bowditch and the grandson of Carl Ludwig. So far as the techniques of the laboratory were concerned this was doubtless more or less true. As to ideas his own imaginative brain was their source. His mind was agile and few workers have been as keen in seeing the many relationships that an experiment usually has. Among Dr. Cannon's files Drinker found one large folder labelled "Questions." Many of these were on bits of paper in his own handwriting. They varied as widely as "Peptic ulcer from simultaneous stimulation of vagus and sympathetic" to "Catnip in relation to sex." What he published was particularly his own. He was generous to his students and like Ludwig, allowed many important researches to appear under the names of others in the laboratory.

Although Dr. Cannon was an extremely industrious laboratory worker he was equally active in many fields only indirectly associated with his researches. He always felt that he was a part of Harvard College as well as the medical school and served on many Cambridge committees. The societies to which he belonged were almost countless. He was particularly influential in the affairs of the American Medical Association, the American Physiological Society, The National Academy of

Science, the American Association for the Advancement of Science, and the Board of Scientific Directors for the Rockefeller Institute.

His chief personal pleasure was roaming the country in a Ford with his family, with whom he was on particularly good terms, trying his hand in sculpture in which he was at least good, and climbing mountains. The Ford must go, it didn't matter much how, just so it arrived.

In 1929 when I spent an afternoon with him and Mrs. Cannon at their home he was complaining of a very distressing skin condition. A year later it was found that he was afflicted with one of the inexorable lymphomatous diseases. Only his most intimate friends knew of the situation and although as Drinker says, "It bit into his humour somewhat," it did not stay his scientific hand and his will to work. The manuscript for *The Way of an Investigator* had its final corrections practically on his deathbed. He died October 1, 1945, at his summer home in Franklin, New Hampshire, of bronchial pneumonia and leukemia.

Few if any American scientists have ever been honored as repeatedly as Dr. Cannon. He received honorary degrees from Harvard, Yale, Wittenberg, Boston University, Washington University, the Universities of Liege, Strasbourg, Paris, Madrid, and Barcelona. He was a Baly Medalist of the Royal Society of Physicians and a recipient of the gold medal of the National Institute of Social Sciences and of the Friedenwald Medal of the American Gastroenterological Association. He was the Croonian lecturer for 1918. He also filled the Linacre, Hughling Jackson, Herter, Harvey, Benson, Caldwell, Kober, Neubold, Welch, and Jones lectureships. At Wisconsin he inaugurated a series of visiting lectures. In the first World War he was a lieutenant colonel and received a Distinguished Service Medal and the British decorated him as Companion of the Bath. The scientific societies of which he was a member are too numerous to mention in full. Fourteen of them are foreign. At home he was a member and past-president of the American Physiological Society, President of the A.A.A.S., a member of the National Academy of Sciences, American Philosophical Society, The American Academy of the Arts and Sciences, and the A.M.A.

He served on countless scientific committees and commissions, such as the Scientific Advisory Committee of the Rockefeller Institute and the Resuscitation Commission.

His laboratory was always a busy place according to Carl Binger with everyone having his well-thought-out problem to do. In spite of all his many other activities Dr. Cannon was a prolific writer. His prose was clear, never over-labored and replete with well turned phrases. His publications embrace a Laboratory Course in Physiology, 1919; Mechanical Factors of Digestion, 1911; Bodily Changes in Pain, Hunger, Fear and Rage, 1915; Traumatic Shock, 1923; Wisdom of the Body, 1932; Digestion and Health, 1936; Autonomic Neuro-effector Systems with Arturo Rosenbleuth, 1937; and the Way of an Investigator, 1945. At least four of these are classical scientific works and their contents have become a part of the general knowledge of all physiologists.

In an address made in 1941 at the fiftieth anniversary of the founding of Stanford University, Dr. Cannon discussed some of the problems confronting medical investigators. He felt that medicine was not subject to the terrific indictment made on science in general by those who could only see the use of new powers for the destruction of man.

The word "geriatric" had not come into common use, but Dr. Cannon noted the increased life expectancy in all highly civilized countries and the concurrent increase in the degenerative diseases. As a good physiologist he asked whether these may be the result in part at least of inactivity, and whether physical training in the later decades of life would be useful. Lack of systematic study is the cause of our lack of knowledge of the disorders of senescence. Tests for fitness are most important. The stress of modern life accentuated by the shift of thirty millions from rural to urban areas since 1900 he believed to be a factor in the greater number of suicides and mental disorders.

The use of drugs was always a fascinating field to Dr. Cannon, particularly those derived from animal sources. He believed each medical school should have a separate department of pharmacology. This subject held an important central position sending its roots into organic chemistry, physiology, bio-

chemistry, and experimental pathology. He dreamed not only of immediate therapy but of a possible control of long-time pathological processes.

To win in these struggles against the common enemies of man there must be freedom for research in the fullest sense. Land frontiers have disappeared but scientific imagination offers again and again suggestions for adventure. Medical research is also international, and gifted men, conquerors of disease, bear the names of all races and nationalities.

Many have accused Dr. Cannon of being an out-and-out pacifist. Certainly he believed that science should be far above the common political bickerings of mankind. Medical science in particular was devoted to the preservation rather than the destruction of mankind. Its devotees then should also be imbued with a spirit of helpfulness and not destruction. He hated war and felt that rational men should not have to indulge in it to settle their disputes. However, he was of too practical a mind not to note that inexcusably unfair and oppressive power did exist in the world. He therefore championed the oppressed in all lands and spent himself in their support. When no other course seemed open, he was willing to fight for them.

Dr. Cannon's first piece of scientific work was undertaken as an undergraduate with Professor C. B. Davenport. It resulted in a joint paper "On the determination of the direction and rate of movement of organism by light" which was published in the *Journal of Physiology* in 1897. In the fall of 1896 when he was a freshman medical student, he and a sophomore, Albert Moser, went to Dr. Bowditch and asked for an opportunity to undertake a physiological investigation. The professor suggested that they test the Kronecker-Meltzer view of swallowing by means of the Roentgen rays which had only been discovered in December of the year before. The suggestion spoke well for the physiological imagination of Bowditch and it offered Cannon the rarely fortunate opportunity of beginning his career on a study which turned out to be of extraordinary interest and importance. As his work developed it became evident that so far as gastro-enterology was concerned he was the scientific successor of Beaumont. Pavlov and Carlson are the two other great names in this field. Four men, Beaumont, Cannon, Pav-

lov, and Carlson have outlined much of the known physiology of the digestive tract.

The first observations made by Cannon and Moser had to do with the passage of pearl buttons down the oesophagus of a dog. This was on December 9, 1896. The experiment was repeated on a rooster on December 14. This was followed by the administration of bismuth subnitrate in capsules to a frog, a dog, and then to a goose. The long neck of this bird was of course attractive and a bird with a high stylish collar to keep the neck elongated was encouraged to swallow capsules and present his oesophageal fluorescent silhouette to the august members of the American Physiological Society at their Boston meeting on December 29, 1896. This was the first public demonstration of a study of the alimentary canal by means of the X ray. Early in January, 1897, they mixed a bread mush with bismuth subnitrate in order to determine the rate of movement along the oesophagus. The goose was used until April 3, 1897, when a cat was studied with the mush-bismuth bolus as well as meat mixed with bismuth. As the food accumulated in the stomach that organ was outlined and on April 23, they first noted peristaltic waves passing over the organ. The original description notes that the waves began near the middle of the stomach and that the pylorus did not open at the approach of every wave. A little later the presence of several waves present at the same time was described. Dr. Bowditch reported these results from his laboratory at the May meeting of the American Physiological Society in Washington in 1897. They were reported by Cannon himself at the regular Society meeting on December 28, 1897, and published in the Proceedings of the Society on April 1, 1898. The completed work appeared under Cannon's name in Volume I of the *American Journal of Physiology* in 1898.

The preliminary reports were with A. Moser, his fellow worker. The complete report was by Cannon alone, Moser apparently having given up the experimental work for his clinical medical studies.

I have mentioned the specific dates to point out what a student in his freshman and sophomore medical years accomplished in less than a year and a half. With characteristic

modesty Dr. Cannon disclaimed much of the credit for the use of the X ray in gastro-enterology. He pointed out that Roentgen himself was aware that the salts of the heavy metals were opaque and that the injection of such materials into the blood vessels and viscera of animals had already been carried out. It was only one further step to mix these salts with food and then follow this through the alimentary canal. Although often in Germany and occasionally even in this country someone credits the bismuth meal to Rieder, Cannon has a clear claim to this method of study, for his first observations in human cases were made in 1898, while Rieder's paper did not appear until 1905.

Dr. Cannon was only twenty-seven years old when his first experiments on gastric motility were made. They were interesting, important and well done and at once attracted international attention and stamped the young investigator as an outstanding physiologist of his day. For fifteen years he continued work on nearly every aspect of gastrointestinal motility. He showed the nature of swallowing, the nearly vertical position of the stomach, the presence of gastric peristaltic waves, the shortening of the stomach as it emptied, hunger contractions, rhythmical segmentation in the intestine, antiperistalsis in the colon, the effect of various nerves on gastric movement, the time of passage for different foodstuffs and the auscultation of gastro-intestinal sounds.

The experimental work during this period was summarized by Cannon in his Monograph of 1911 on "The Mechanical Factors of Digestion." Of the conclusions presented there, all have stood the test of time except his acid theory of pyloric control. Although all details are not yet entirely clear it is now believed that the sphincter is more closely related to entero-gastric pressure than to the acidity of gastric contents. The monograph was appropriately dedicated to Professor Bowditch and is an excellent example of carefully described and documented scientific reporting.

Michael Foster once said of Claude Bernard that he had that "chiefest sign of genius in inquiry," which was "to leave the road at the right turning and to follow a bye-path which brought him to a great result." Something of the same may be

said of Cannon. The sign which directed him, not into a bye-path, but into a new highway was the observation that when one of his experimental animals grew restive or was provoked to anger, the gastro-intestinal movements ceased. Male cats were particularly obstreperous and even Cannon, the most skillful manipulator of cats, could seldom quiet them. Females were therefore usually used but if they too grew irritable from the experimental conditions, all gastro-intestinal activity ceased. To many a worker this would have been only a matter of inconvenience and evidence of a faulty experiment or the need of some other experimental animal. Cannon, however, like Bernard held there never was a "bad" experiment. The fact that it did not turn out the way it was expected was no proof that the laws of nature were not being followed. It was only a challenge to unravel some kind of an unknown complex situation.

Analysis of these phenomena, in colloquial phrase, why a cat's stomach and intestine quit working when the animal gets mad, kept Cannon busy for the next twenty years, and its ramifications, the rest of his life. He noted that rage, anger, fear, asphyxia, and pain not only abolish gastro-intestinal movements but also the secretory activity of the digestive glands. On the other hand these same states resulted in a strengthened heart, an increase in blood pressure, a greater respiratory exchange, dilatation of the pupils, elevation of the hair, increased amount of sugar in the blood, reduced muscular fatigue and decreased coagulation time. He next noted that any one of these results could be secured by stimulation of the sympathetic nerve to the appropriate organ. It was evident, however, that normally the sympathetic nervous system functioned as a unit and not in any uncoordinated piece-meal fashion. Cannon was also well aware that the injection of adrenalin would produce the same effects as sympathetic stimulation. The previous work of Dreyer who showed that the medulla of the adrenals was a secretory organ under sympathetic control now began to have new meaning. At this point Cannon formulated what is generally spoken of as his "Emergency Theory of Adrenal Secretion." Briefly stated his idea was that in times of physiological stress the sympathetic

nervous system is brought into action to enable the body to perform extra work and to prevent changes in its internal environment. In this physiological series of adaptations, the secretion from the medulla of the adrenals not only brings about responses characteristic of its action when injected, but it reinforces the entire sympathetic system. It is so intimately connected with this division of the autonomics that we regularly speak of the sympathetic-adrenal system.

The idea that the clue to all physiological processes was to be found in an attempt to preserve the status quo, that is, the internal environment of the organism, was first clearly expressed by Claude Bernard. It is an inherent point of view in the work of many English physiologists, particularly E. H. Starling. It was Cannon's part to see that the bodily changes in the emotional states were also for the same purpose, that is, bodily preservation and that they were brought about and coordinated by sympathetic-adrenal activities. The theory as a whole was developed and presented by Cannon in his monograph of 1915 on *Bodily Changes in Pain, Hunger, Fear and Rage*. A second edition in 1929 contained new chapters on thirst, nervous centers for emotional expression and a critical review of the James-Lange theory of emotions.

With the exception of his work on shock, to which we will refer shortly, Cannon spent nearly all of his scientific life in investigating aspects of physiology which put his emergency theory to the test or explained the mechanisms involved.

Defense of the part that he believed the adrenals played in the emergency mechanisms led Dr. Cannon into a long and bitter controversy with Drs. Stewart and Rogoff of the Department of Experimental Medicine at Western Reserve University. The most important point at issue was whether an increased secretion of adrenalin could be shown in conditions calling for unusual bodily effort. The methods of collecting blood from the adrenals and the value of biological assays played a large part in these controversies. Stewart and Rogoff asserted that adrenalin output was fairly constant, while Cannon with equal insistence asserted it might be increased as much as tenfold above their resting level.

Although for a number of years the annual debate between the two parties served for entertainment as well as instruction, it gradually became so bitter that it was unpleasant for all concerned. I remember hearing Dr. Macleod advise Dr. Cannon not to continue the polemic until he had results from an indicator of adrenal secretion that was entirely above criticism.

It is pleasant to recall that Dr. Cannon credits our own laboratory with the suggestion that the denervated heart might be used for such an indicator. Fright, rage, pain, anesthesia, and asphyxia all resulted in a greatly increased heart rate within ten seconds. This was prevented by removal of the adrenals. From this time on Cannon seems to have had the best of the arguments and the emergency theory seems today to be on safe ground and generally accepted.

It was characteristic of Dr. Cannon that side issues always aroused his interest. Although on removal of the adrenals there was no longer a prompt or rapid increase in heart rate, there was still a slowly developed acceleration during emotional states. Although this acceleration amounted to only a few beats it was not overlooked by Dr. Cannon. He found it persisted after removal of the upper thoracic chain, but was prevented by blocking the venous return from the lower part of the body. A chemical substance was therefore liberated from the sympathetic endings particularly in the smooth muscle of the skin, which resembled adrenalin. He named it sympathin.

In support of his emergency theory Dr. Cannon realized that correlation of the various organs concerned was necessary. This could only be brought about through the nervous system. Emotions were not due primarily to secretion of adrenalin but the secretion potentiated the activities induced by the nervous system itself. By decortication and hypothalamic sections he and Britton found that the center governing emotional expressions was located in the caudal nuclei of the hypothalamus. So long as this center was connected to the sympathetic system a condition of "sham rage" could be produced. Such a cat showed all signs of anger, spitting, scratching, dilatation of pupils, and elevation of hair. Further experiments proved that it was the sympathetic system that mediated these effects. On the removal of the entire sympathetic chain the animal was

able to exist very comfortably so long as it had no emergencies to meet. Cannon thus showed the mechanisms by which his emergency reactions were carried out. Cannon was imaginative and a great speculator, but he never for one moment forgot that after all the real problem of a physiologist is to answer "how" a process is carried out.

In 1917 Dr. Cannon entered the services as a medical officer in the American and British Expeditionary Forces. After reaching France he soon engaged in an intensive study of traumatic shock. This condition was the cause of a high percentage of deaths among the wounded. Cannon made observations at the front under actual bombardment, in the English laboratories particularly with Bayliss and at the laboratory for surgical research at Dijon. The result of his work was published in the volume *Traumatic Shock* in 1923. This monograph was of inestimable value in crystallizing current views on shock. Cannon's theory of the cause of shock was that of a "toxic exemia," a loss in the effective circulation of blood due to toxic products from the injured tissues. A careful analyses of shock studies during the last twenty-five years shows that our ideas of the fundamental nature of shock are about where Cannon left them, although emphasis has somewhat shifted. Workers now generally agree that a decrease in the effective circulation is the real cause of shock. Cannon and Bayliss did not underestimate this point and suggested the injection of acacia solution as the best means of combating blood loss, since blood itself was rarely available.

In 1940 when the dangers of a second world war loomed large on the horizon the National Research Council made Cannon chairman of a Committee on Shock and Transfusion. Although this time his work was largely advisory he had the pleasure of seeing Max Strumia and Edwin J. Cohn develop methods of blood preservation which made possible the treatment of shock even at the front line stations. Shock suddenly ceased to be the great threat to life that it was in the first world war.

It should not be forgotten that the advances in practical substitution therapy in shock really stem from the work of Bayliss and Cannon in 1917 and 1918. As to the fundamental cause

of the decrease in the effective circulation, recent workers have generally leaned toward the idea of local losses into the traumatized and adjacent tissues. The toxic theory of Cannon has however been by no means entirely abandoned. At the present time Ephraim Shorr in particular has isolated definite toxic depressor substances from shocked animals. The future may well show that Cannon was not far wrong in his overall conception of the various aspects of traumatic shock.

One of the most interesting biographies of all time, so far as biologists are concerned, I am sure is Dr. Cannon's *Way of an Investigator*. He does not spend too much time on personal details, but he does have much to say about his interests, scientific endeavors and methods of research. Coupled with this he gives his social views and the relations of a scientist to the social and political world in which he lives. All this is done with humour and insight but with extreme modesty. If it ever entered Cannon's mind to question whether he was truly great, it never reached the attention of his friends or colleagues. One cannot read the *Way of an Investigator* and not be convinced but that Cannon had the spirit of one who was truly great.

Carl Binger who knew Cannon well says that he had a Gaelic respect for logic and Irish wit. Cannon loved to quote the words of Jim Skillen, his faithful janitor who said at the birth of the first Cannon child, "In a family with man child there are three fools." It was this same Jim Skillen who referred to the celebrated embryologist, Dr. Minot who had no children, as "a theoretical embryologist." Dr. Cannon lover nonsensical jokes. Once while passing through Milwaukee he called up his old comrade Dr. Jack Yates in broken Swedish dialect, and bothered the busy Dr. Yates no end, until he got wise.

It would be a pleasure to review the entire book on the *Way of an Investigator* but only a few chapters can be mentioned here. In a chapter titled "Fitness for the Enterprise" he discusses the essential qualities of the investigator. Inquisitiveness, imagination, critical ability and technical training are necessities. Patience, willingness to take pains, generosity, a humble attitude and thorough honesty should all be added to the list.

The favorable factors influencing research are freedom of action, feeling of security and absence of pressure to produce

within a given time. Teachers can well afford to spend part of their holiday in the joy of research. One of the most unfavorable factors for medical research are the attempts to abolish animal experimentation. Dr. Cannon wrote many articles defending the use of animals and spent much time in opposing ruthless interference in a type of investigation that has saved vast numbers from suffering and premature death.

In one chapter he discusses the "Role of Hunches," those flashes of insight that come in unpredictable moments. A typical case is a thought that springs into consciousness when the investigator is not working on this subject. Cannon had many examples in his own experience, most of these occurring during wakefulness at night. This is the only credit he finds that can be awarded to insomnia. It was in such periods of sleeplessness that he suddenly formulated the emergency theory and visualized his coagulometer. Scientific history has many similar examples. Loewe dreamed about a method to detect chemical intermediation, was unable to read the notes he hastily wrote that night, but fortunately had his flash of insight repeated the second night. He lost no time in arising and made his notes legible. Among the unfavorable conditions for hunches Cannon listed mental and physical fatigue, petty irritations, noise, worry over domestic or financial affairs, strong emotions, and work under pressure. Among the favorable conditions were a clear definition of the problem, eager desire for its solution and a large store of related information. As always the prepared mind is the successful one.

Just as a hunch may occur at an unexpected moment so unexpected or lucky discoveries may be made by experimentalists. In 1754 Horace Walpole in a chatty letter to his friend, Horace Mann, introduced a new term to cover this situation. In a certain fairy tale the princes of Ceylon, the ancient name of which was Serendip, were always making discoveries by accident. This faculty Walpole called "Serendipity," a word which greatly intrigued Dr. Cannon. Galvani's discovery of electrical phenomena in muscle and Claude Bernard's finding of the vasomotors are classical examples of physiological discoveries by what might be called lucky accidents. Many others might be pointed out. In recent days we are familiar with the development of penicillin which began with the accidental contamination of a bacterial culture with a mold. The chance

observation of Fleming was turned into practical therapeutics by the long hard labors of Howard Florey. Cannon cites in his own case two examples of serendipity. One was the stoppage of gastric and intestinal movements in times of anxiety. The other was the acceleration of the nervously isolated heart on stimulation of sympathetic fibers in a distant part of the body. Most workers would have discarded these experiments as some kind of failure, but they really were lucky accidents, at least their observation was accidental.

Cannon's generosity to his fellow workers has already been mentioned but it may be further stressed. He was what might be termed a "synthetic" physiologist. His emergency theory was built up from the detailed observations and experiments on single organs. While he was busy with problems of integration he says he had the good fortune of having with him Alexander Forbes and Hallowel Davis, both expert investigators with analytical methods. Not only were these men of the greatest help, but the variety of endeavor made the Harvard physiological laboratory a magnificent teaching institution.

Among his working principles Dr. Cannon places as his first article of belief that what happens to our bodies is directed toward a useful end. A search for utility may therefore result in new discoveries. The view that there are organic adjustments for the good of the whole organism involves the conception that these activities are "directed," which is "teleology." This turns a physiologist from "how" to "why" and so may lead to a useless realm of speculation. Many scientists hesitate to take this step even if they do agree with von Bruecke who remarked, "Teleology is a lady without whom no biologist can live, yet he is ashamed to show himself with her in public." Cannon was quite willing to use teleological reasoning, though with caution. In this way he suspected inhibition of the colon as food entered from the small intestine, stimulation of the adrenals in excitement, and hyperglycemia in fits of rage. Teleology is not a matter of intelligent foresight but a response that has a survival value.

Another article of Dr. Cannon's scientific faith was that structure and function are inseparably related. Where structure is complicated, function is likewise complicated.

As for homeostasis, if a state remains steady it does so because any tendency toward change is met by increased effectiveness of some factors which resist change. When a factor is found that can shift a homeostatic state in one direction, it is reasonable to look for automatic control of that factor.

Much of the human interest in the *Way of An Investigator* cannot be retained in the retelling. Cannon's love for his friends and students, his belief that scientific methods could be used to solve social and political problems, his estimate of cooperative research, his desire to make science understandable, all make up interesting and important paragraphs or chapters.

On her deathbed, Walter Cannon's mother gave him a single piece of advice. It was the brief terse sentence, "Be good to the world." It may be that his hearty, generous nature allowed him to follow this admonition without conscious effort. Be that as it may, now that he is gone, the words seem to epitomize the purposes of his life.

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FAILURE TO DEMONSTRATE A VIRUS IN VAGUS NERVES OF PATIENTS WITH CHRONIC RECURRENT DUODENAL ULCER*

EDDY D. PALMER AND REGINALD L. REAGAN

An infectious basis for duodenal ulcer has by no means been entirely disproved, but at the moment only one theory of direct infectious association remains active. Loforte Gonçalves of Brazil has at intervals since 1946 presented evidence which suggests to him that a herpes simplex-like virus, which becomes established in the vagus nerves through a break in the gastroduodenal muscosa, may be responsible for initiating recurrences of duodenal ulcer in man. It is important to note that Gonçalves has proposed an explanation for recurrence, not for causation.

In an attempt to confirm his work, virus studies were carried out on the segments of abdominal vagus nerves removed during surgical treatment of patients with chronic recurrent duodenal ulcer.

Material. Eight men with duodenal ulcer and with moderate to severe courses, in terms of chronicity and number of recurrences (Table 1), were studied. The diagnosis in each had been

TABLE 1

Patient data

Patient	Age	Length of ulcer history, years	Recurrences necessitating hospitalization
1	40	8	5
2	36	8	6
3	38	5	5
4	38	14	4
5	42	20	4
6	41	7	6
7	35	11	5
8	54	8	3

proved time after time by roentgenologic means and was further confirmed at operation. Because of failure of medical measures

*From the Gastrointestinal Section, Walter Reed Army Hospital, Washington, D.C., and the Virus Laboratory, University of Maryland, College Park, Maryland. Received for publication December 1, 1952.

to prevent excessive disability, gastroenterostomy and transabdominal vagotomy were done.

Technic. From each of the eight patients segments of abdominal vagus nerve totalling approximately 3 cm. in length were removed. The nerves were placed in 0.5 ml. of sterile physiologic saline solution and immediately frozen. Two hours later 0.5 ml. of physiologic saline solution was added, and the nerve segments were triturated with the abrasive help of alundum. Following centrifugalization at 1500 r.p.m. for 35 seconds, 0.2 ml. of suspension was inoculated intracerebrally (midline) into each of three young rabbits. The animals were examined twice daily for fourteen days, for neurologic signs and other evidences of disease.

Results

All of the rabbits remained healthy throughout the 14-day observation period. At no time did any of the animals demonstrate signs of central nervous system involvement, such as those which follow intracerebral inoculation with the herpes simplex virus.

Conclusions. No information was obtained which might encourage further search for a herpes simplex-like virus in the vagus nerves of persons with chronic recurrent duodenal ulcer.

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ROLE OF THE SPLEEN IN RESISTANCE TO EXPERMENTAL TUMORS*

MORRIS POLLARD AND ROBERT BUSSELL

The lymphatic system appears to possess an important role in the mechanism of tumor immunity in rodents: Murphy (1) and Clemmensen (2) depleted this system in rats with x-irradiation and thereby reduced their resistance to experimental tumor heterotransplants. Similarly treated rats (3) as well as rats treated with cortisone (4) have supported the growth of malignant tumors of human origin. Inoculations of alien rodent lymphatic tissue induced resistance to homotransplantable tumors after 4–7 days (5). The successful propagation of human tumors in the anterior chamber of the guinea pig eye and in brain tissue might be attributed to the absence of an immune mechanism in those tissues (1, 6, 7). When avian embryos were supplied with adult spleen tissue a tumor-resistant state resulted (1). Interspecies transfer of avian leukosis was successful only when such species were inoculated *in ovo* at an age when the immune mechanism was absent (8).

When rodent tumors regress spontaneously, such animals are subsequently refractory to reinoculation (9, 10); however, tests of the specific oncolytic effect of antiserum from such animals are still indecisive (11, 12).

Recent tissue culture studies at this laboratory have revealed a tumor controlling function of spleen tissue. Such observations have revealed reactions simulating homoplastic and heteroplastic transplantations of mouse and rat sarcomas, and they may provide an explanation for the success or failure of such transfers. The technique used also provides a means of observing an "immune" reaction between incompatible tissues.

Methods. The rodent tumors and the tissue culture roller tube techniques employed in this study have been described in a previous paper (13). Four to five small pieces (1.0 cu. mm.) of fibrosarcoma from C3H mice[1] were explanted in coagulating

*From the Virus Research Laboratory of The University of Texas Medical Branch. Received for publication November 4, 1952.

[1] Obtained from Carl A. Nau, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston.

chicken plasma on the wall of a tube. To the opposite side of the tube an approximately equal amount of spleen tissue was similarly explanted. All tissue fragments were washed in 4–5 changes of balanced salt solution (Hank's) prior to explantation. The sources of spleens used are explained as follows:

1. "Homologous" mouse spleen was taken from the tumor-affected animal.
2. "Normal" mouse spleen was taken from C3H or from CFW mice which were previously unexposed to tumor transplantation.
3. "Immune" C3H mouse spleen was taken from mice in which progressively growing C3H tumors had failed to develop or had retrogressed.
4. "Immune" CFW mouse spleen was taken from mice that had received one or more inoculations with tumor tissue from C3H mice without any evidence of tumor development.

An anaplastic sarcoma[2] in Sprague-Dawley rats was similarly studied with spleen tissue from the same rat ("homologous") and from rats in which the tumor had retrogressed spontaneously with subsequent resistance to reinoculation ("immune"). Control tubes in all instances contained only tumor or spleen tissue.

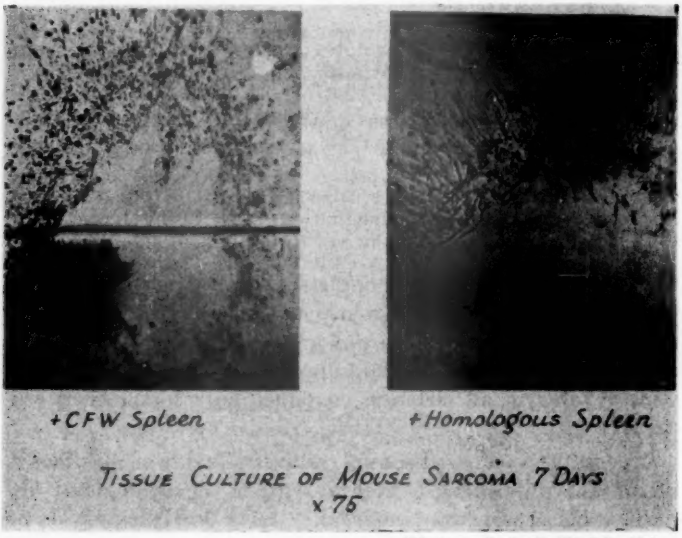
The cultures, each with 1 ml. nutrient fluid, were incubated at 37° C. while being rotated at 6 r.p.m. at an angle of 5°. Each explant was examined daily under low magnification. The nutrient fluids were adjusted daily to optimal pH according to the color of the fluid. The fluids were changed at 3–5 day intervals.

Results

Mouse tumor implants grew rapidly, and after 24–48 hours a distinct "halo" of fibroblast-like cells was visible around each explant. Areas of plasma liquefaction frequently developed within the "halo," and the cells therein were round. Tumor explants, in combination with the "homologous" C3H spleen, grew equally as well as above during the 10-day observation period. In combination with spleens from "normal" CFW

[2] Originally induced by methylcholanthrene at the M. D. Anderson Foundation for Cancer Research, Houston, Texas.

mice, the tumors grew well for the first 3–4 days; however, by the 4th–5th day, degenerative changes appeared in the tumor tissue, particularly in the "halo." Such degenerative changes proceeded centripetally to destruction of the entire explant by the 7th and 8th days (Fig. I). The latter sequence was also



(Photographs by F. W. Schmidt)

FIGURE I

observed in some of the tumor + "normal" C3H spleen cultures (Fig. II). In most of the latter combinations, the degenerative effect was incomplete and then was reversed. In cultures of tumor with "immune" CFW spleen, a retarded tumor growth was observed on the 1st and 2nd days and its destruction progressed to completion by the 6th–7th day (Fig. III). A tumor suppressant effect was exerted by "immune" C3H spleen explants, the destructive effect appearing during the first two days after explanation (Fig. IV).

The rat sarcoma tissue grew rapidly in the form of a mass of round cells extending from the periphery of the explant. Such a tumor grew exceedingly well with the "homologous"

spleen; but, with "immune" rat spleen, the tumor growth was much retarded on the 1st and 2nd days. Most of the tumor tissue of the latter combination was destroyed by the 6th–7th day,

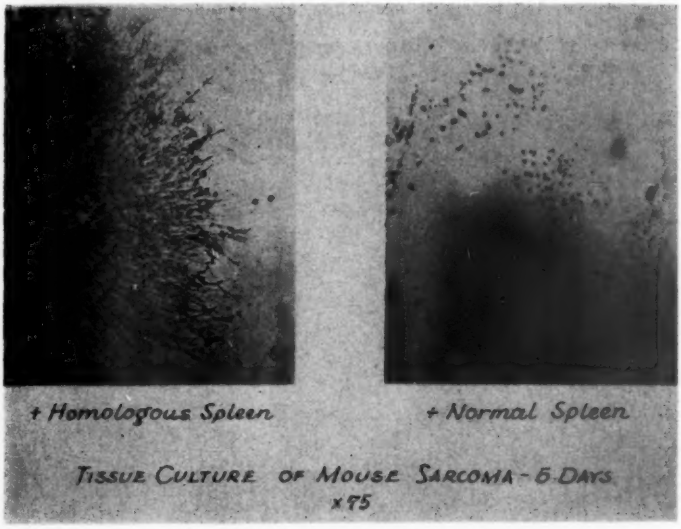


FIGURE II

contrasting sharply with the rapidly growing former combination (Fig. V).

In combination with the tumors, the "homologous" splenic explants grew out as fibrocytes; however, the growth of the tumor-destroying splenic explants consisted mainly of free round or ovoid cells (Fig. VI).

When degenerative changes appeared in the growing tumor explants the periphery of the "halo" appeared granular. The cell nuclei became pyknotic and cell outlines became hazy and fragmented. The "halo" became attenuated progressively in centripetal direction, leaving a wake of some poorly identified cellular and nuclear debris. Eventually the original explant became opaque. Coincident with these degenerative changes in tumor tissues, distinct rounded cells appeared in the area sur-

rounding most of the spleen explants (Fig. VI). These latter cells showed no direct attachment to the explant.

Discussion. The results described above would suggest that antagonistic factor has been engendered against an oncotic mechanism by reactant spleen tissue. Whether this effect is

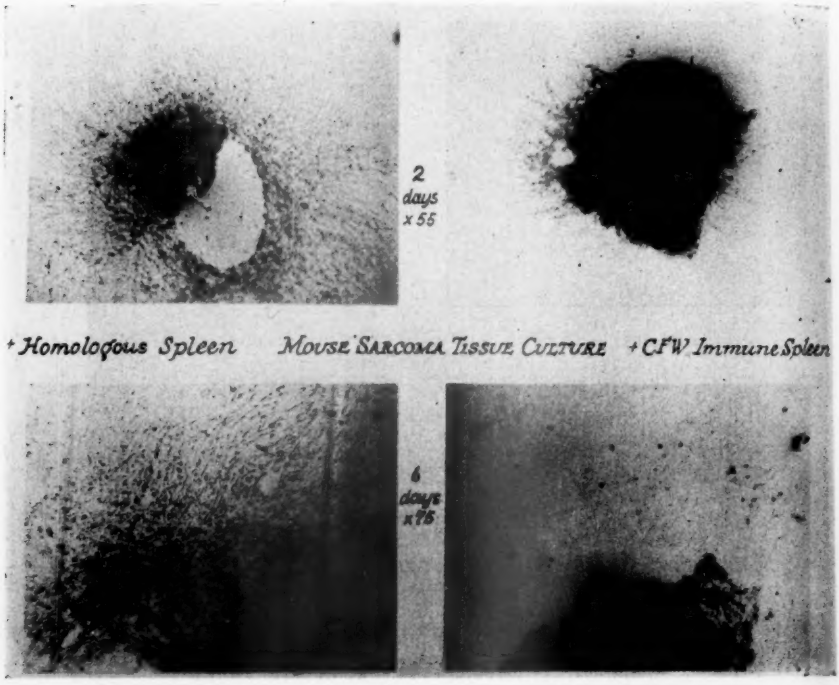


FIGURE III

in the nature of a conventional antibody is as yet uncertain. An immune mechanism might explain the tumor refractory status of several of our rats after their tumors had spontaneously retrogressed. It is apparent that antagonism to tumor is manifested with a heterozygous spleen-tumor combination and that this effect can be accelerated by previous exposure of the refractory animal to the tumor. Experimental data of others (14, 15, 16) would suggest that conventional antibody production usually

occurs 4–5 days following exposure of the host to antigen. The degenerative effect in tumor explants, as described above, was visible after a latent period of 4–5 days and this period was greatly shortened with the "immune" spleen.

The cytological characteristics of the spleen outgrowth would appear to reflect its ability to destroy the tumor explants. Lymphoid cells (plasma cells) have been associated by others (1, 17,

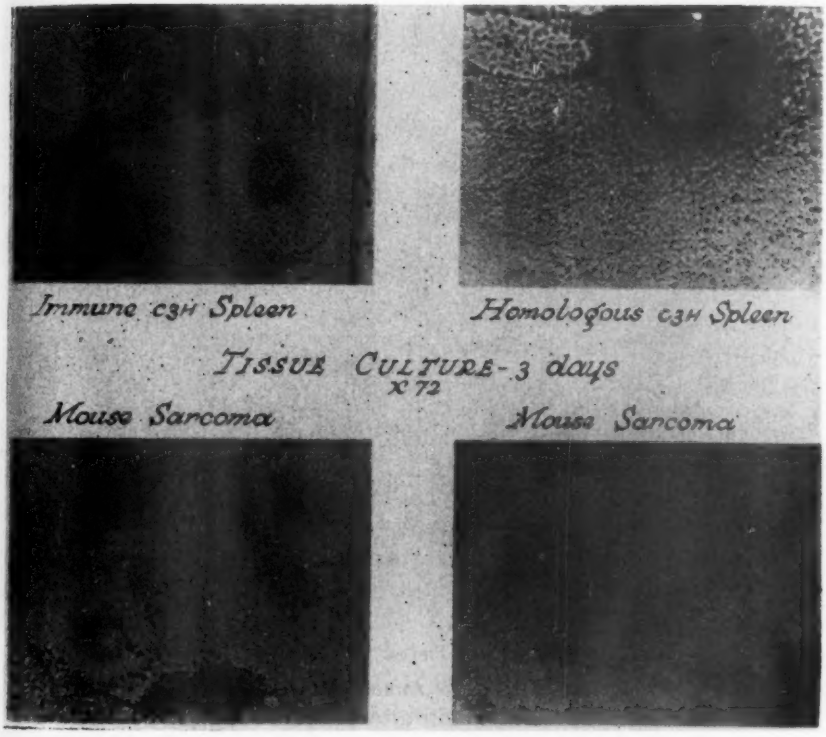


FIGURE IV

18, 19) with antibody production and in our observations the lymphoid type of cell predominated around the spleen tissue associated with the degenerating tumor tissue. It is possible that the splenic reaction observed may exemplify that of all

reticulo-endothelial tissue in the host. The lymphoid cells surrounding the "immune" spleen explant may be fibrocytes which have rounded-up when the plasma in which they are embedded undergoes liquefaction. Spleen tissue, without degenerative influence on tumor tissue generally grew as a fibrocytic halo and the lymphoid type of cell was scarce. Shabad

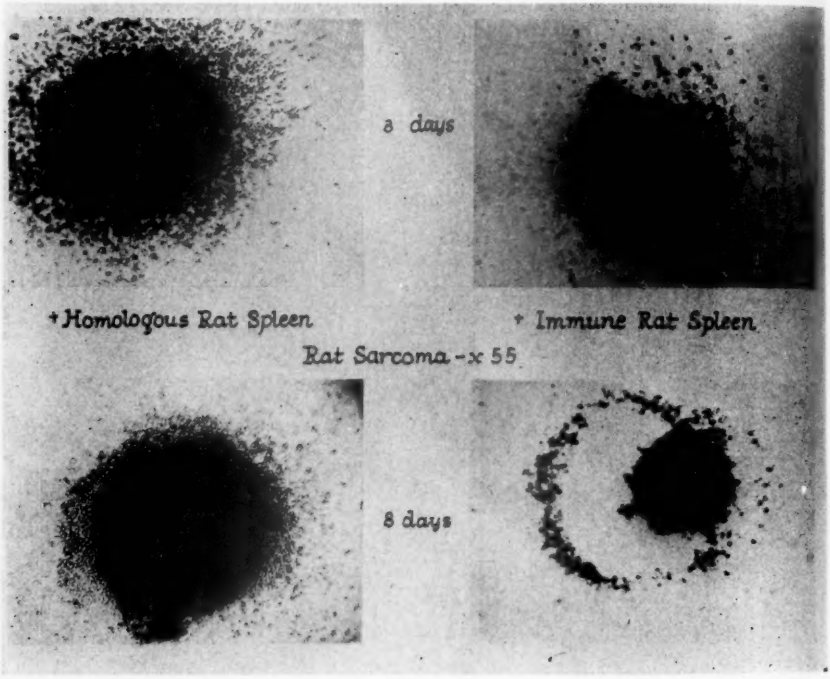


FIGURE V

briefly reported (20) on the tumor enhancing effect of homologous spleen tissue. A definite growth stimulus was not demonstrated in our studies with tumor-homologous spleen combination; however, growth of tumor in such combinations was as definitely not interfered with. The only notable difference between tumor cultures with immune and with homologous spleen tissues was the cytology of spleen explant outgrowth.

Although an immune phenomenon is thus demonstrable *in*

vitro, this destruction of a heterotransplant by recipient tissue is not an unexpected finding and offers little to explain the behavior of tumor tissue as homotransplants. The occasional

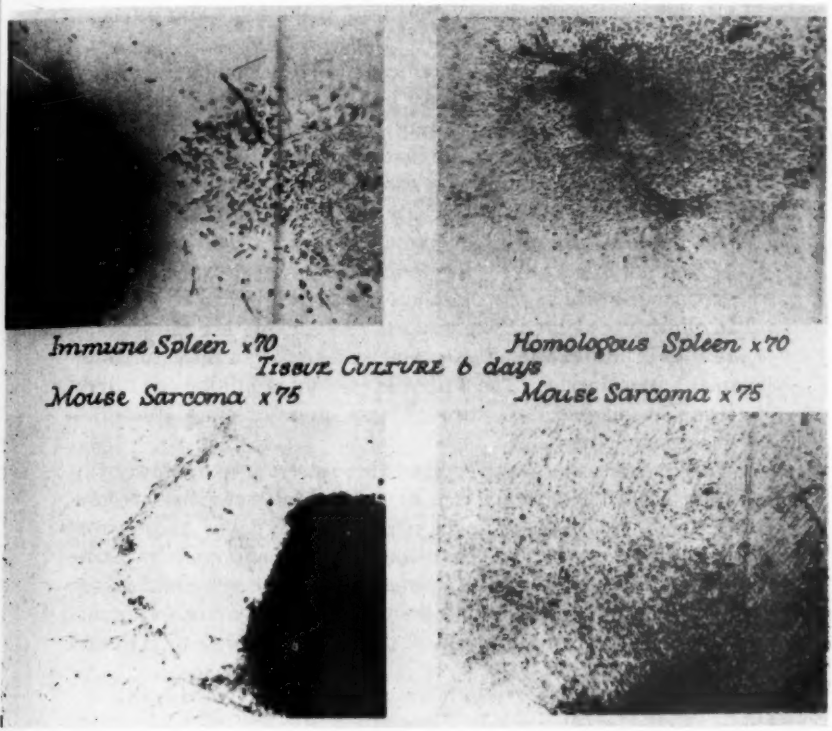


FIGURE VI

susceptible animal which fails to develop a tumor or in which the tumor retrogresses, might thus manifest some genetic deviation; however the strains here used have been inbred in pure line for many years and no reason for such deviation is apparent. Rats and mice which have resisted reinoculations with homologous tumors exert, through their spleens, a tumor antagonistic factor. The passive effect of residual serum is minimized by the thorough washing of spleen tissue prior to ex-

plantation, and by the progressive deterioration of tumor tissue. This simulates the antitumor effect of CFW immune spleen. What provokes such an immune response on the part of a recovered and resistant host? The data presented here and that of others indicated above might lead to the following interpretation:

When tissues are transplanted from one genetic strain to another, incompatibilities may be revealed by degeneration of the transplanted tissue. Tumors grow progressively in hosts which are homozygous to the donor; however, occasionally such a tumor retrogresses leaving the host refractory to reinoculation. An immunological anomaly may thus result in which the host is thus "immune" to tumor tissue to which it is closely related antigenically. The tumor may be provoked by an exogenous agent of such tenuous relationship as to be tolerated in the invaded host. The sudden reversal of this relationship results in absorption of the tumor and in immunity to subsequent inoculations. The tumor-host compatibility may therefore be perpetrated through an immunological defect—genetically linked.

Once the tumor tissue is exposed to spleen of an heterozygous (nonsusceptible) strain, a distinct immune response is precipitated, following a latent period of 4–5 days. As observed herein, an immune mechanism has been observed by tissue culture methods in which spleen tissue is instrumental in the destruction of incompatible tissue. Such a phenomenon occurs also when an homozygous host develops resistance to a tumor, causing it to retrogress.

Summary

By tissue culture techniques it has been demonstrated that spleen tissue exerts a controlling effect on the growth of rodent tumors, as follows: spleen tissue from the "homologous" host had no deteriorative effect on the tumor; that from a recovered homozygous host had a rapid destructive effect; and that from a genetically unrelated rodent source exerted a destructive effect after a latent period of 4–5 days. If the latter host had been previously "immunized," its spleen manifested an accelerated effect in 1–2 days. Our interpretation is that the tumor or its provocative agent may be only slightly exogenous to

the host and that a genetically-linked immunological defect on the part of the host thus permits progressive growth of the tumor.

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COMPLEMENT FIXATION REACTIONS IN SERUM HEPATITIS*

MORRIS POLLARD AND ROBERT BUSSELL

Early descriptions of viral hepatitis failed to differentiate those cases occurring in epidemics from those occurring less frequently and associated with a previous inoculation of blood or with a blood-contaminated instrument. Both syndromes are similar in many respects. They are poorly differentiated by liver function tests and by the microscopic examination of liver biopsy specimens (Findlay, *et al.*, 1944). The two were considered dual manifestations of the same disease until Havens (1945) demonstrated that recovery from one engendered no immunity against the other. Prior investigations (Havens, 1945 and Findlay, *et al.*, 1945) demonstrated that the agent causing infectious hepatitis (IH) could be demonstrated in the nasopharynx, stools, and blood of cases of the disease. On the other hand the Serum hepatitis (SH) agent was found only in the blood (MacCallum, 1944). IH is therefore thought to be disseminated by fecal and oral contamination of the environment, SH by artificial passage.

SH first gained prominence during World War II, following the administration of yellow fever vaccine which had been prepared with SH contaminated serum (Sawyer, *et al.*, 1944). In the course of extensive investigations to determine the character of this illness, serum from acute cases of the disease fixed complement with a pool of serum collected from convalescent cases of the disease. The complement fixation (CF) studies did not establish the specificity of the reaction, since only serums considered "normal" were studied as controls (Sawyer, *et al.*, 1944). Precipitation tests were indecisive and results were attributed to liver damage. SH and IH viruses have recently been propagated in tissue cultures containing non-human tissues.

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These cultures have yielded skin test antigens of retrospective diagnostic value (Henle, *et al.*, 1950, 1952).

Materials and Methods. In the course of studying the problem of SH a series of serum specimens were collected from patients hospitalized because of hepatitis following a transfusion or other inoculation. On this basis and on the basis of history, serum bilirubin levels, thymol turbidity tests, and microscopic changes in liver biopsy specimens, they were diagnosed as SH. Serum specimens were also collected from cases clinically diagnosed as IH, from clinically confirmed cases of obstructive juandice, and from serologically and clinically confirmed cases of infectious mononucleosis (IM). The obstructive juandice cases were due to carcinoma of the pancreas, lymphoblastoma in the bile duct, or congenital biliary atresia; and were so diagnosed either by exploratory laporotomy or at autopsy. Charts I and II indicate the diagnosis of each case studied. The clear serum specimens were stored at −20° C. until used and all were heated at 61° C., for 20 minutes before used in the CF test.

CHART I

Antigen	Dx	Convalescent Antiserum 1:5	Dx	Days from Onset	CF Reaction	A/C
Serum of Bergmann	SH acute	Smith	SH*	23 d.	++++	neg.
		Okelberry	SH	35 d.	++++	
		Holligan	SH	54 d.	neg.	
		Clisby	SH	13 d.	++++	
		Bubanik	SH	38 d.	neg.	
		Michaelis	SH	75 d.	+++	
		Routledge	SH	150 d.	++++	
		Guerra	IH	25 d.	neg.	
		Guerra	IH	75 d.	neg.	
		Williams	IH	60 d.	neg.	
		Leyva	IH	68 d.	neg.	
		Davis	IH	67 d.	neg.	
		Klein	IH	48 d.	neg.	
		Patterson	OJ		neg.	
		Mierzwik	OJ		neg.	
		Silva	OJ		neg.	
		Jefferson	OJ		neg.	

*SH—Serum Hepatitis
IH—Infectious Hepatitis
OJ—Obstructive Jaundice

CHART II

Positive SH Antiserum	Antigen	Dx	Days from onset	CF Reaction	A/C
Okelberry	Alivarez	IH	7 d.	neg.	neg.
	Guerra	IH	2 d.	neg.	neg.
	Davis	IH	67 d.	neg.	neg.
	Akiba strain	IH	acute	neg.	neg.
	Bubernik	SH	31 d.	neg.	neg.
	Holligan	SH	55 d.	neg.	neg.
	Even	SH	35 d.		
	Colunga	SH	20 d.	neg.	neg.
	Patterson	OJ		neg.	neg.
	Mierzwik	OJ		neg.	neg.
	Silva	OJ		neg.	neg.
Smith	Alivarez	IH	7 d.	neg.	neg.
	Guerra	IH	2 d.	neg.	neg.
	Klein	IH	48 d.	neg.	neg.
	Davis	IH	67 d.	neg.	neg.
	Gable	IH	180 d.	neg.	neg.
	Longoria	IH	33 d.	neg.	neg.
	Caskey	IH		neg.	neg.
	Akiba strain	IH	acute	neg.	neg.
	Even	SH	35 d.	neg.	neg.
	Colunga	SH	20 d.	neg.	neg.
	Silva	OJ		neg.	neg.
	Patterson	OJ		neg.	neg.
	Coulon	OJ		neg.	neg.
	Tucker	Biliary Atresia		neg.	neg.
	Garcia	Atresia		neg.	neg.
	Smith	Toxic Hepatitis		neg.	neg.
Michaelis	Alivarez	IH	7 d.	neg.	neg.
	Guerra	IH	2 d.	neg.	neg.
Clisby	Davis	IH	67 d.	neg.	neg.
	Goble	IH	180 d.	neg.	neg.
	Longoria	IH	33 d.	neg.	neg.
	Akiba strain	IH	acute	neg.	neg.
	Patterson	OJ		neg.	neg.
	Silva	OJ		neg.	neg.
	Coulon	OJ		neg.	neg.

Two SH specimens were classified clinically as acute cases and so their serums were used as antigen. The rest of the serums from SH cases were assumed to be convalescent specimens and these were used as antiserums.

In setting up the CF test, 0.2 cc. each of 1:1 dilution of acute serum, of 1:5 dilution of convalescent serum, and of complement (2 units) were mixed and incubated overnight at 4° C. 0.2 cc. each of amboceptor and of 2 per cent washed sheep rbc. were then added and the result recorded after incubation at 37° C. for 30 minutes.

The serum specimens from the IH and the obstructive juandice cases were also employed both as antigen and as antiserum in the same manner as described above. Serum containing the Akiba strain[1] of IH virus was also used as antigen with the antiserums listed above.

Results

One of the two acute SH serum specimens fixed complement with the antiserum from 5 of 7 convalescing cases of SH (Table 1). The 2 negative serums contained no detectable CF antigen when tested with the positive antiserums. The negative acute serum was subsequently found to have SH-CF antibody. The SH antigen fixed complement up to a dilution of 1:4, but was used in 1:1 dilution (4 units) for each test. The CF reaction was negative when set up with serums from convalescing IH, from obstructive juandice, and from IM cases. Acute serums from cases of IH and of obstructive juandice did not fix complement with the SH convalescent serums.

The SH antigen tolerated heat treatment at 61°C. for 20 minutes and survived storage at — 20° C. for at least 1 month.

Antibody titrations were not made because of the limited quantity of SH antigen available. SH antibody was demonstrated in serum specimens collected from the 13th to the 150th day following the onset of the disease and at no time in serums of IH, of obstructive juandice, and of IM.

Discussion. We have no proof that the specific antigenic properties of the acute SH serum was due to SH virus since human inoculation experiments were not performed. In spite of this the serum demonstrated antigenic properties which were specific for SH and differentiated it from unrelated icterus due either to another infectious agent or to noninfectious obstruction. The two cases of hepatitis, negative with SH antigen, may

[1] Kindly supplied by Dr. Werner Henle, University of Pennsylvania.

have been due to an unrelated SH virus, to an IH agent, or to a delayed antibody response to SH. We have not yet demonstrated a specific CF test for IH. There is as yet no indication of multiple strains within the SH and IH prototypes of virus. Therefore, the identity of the above two cases must remain undefined.

It has been estimated that individual specimens of SH contaminated plasma may contain at least 25,000 infective units per ml. (Drake, *et al.*, 1952). It is conceivable that such a concentration of virus could serve satisfactorily as CF antigen. It is hoped that further studies of the CF antigenic content of SH infected serums will serve to confirm these findings and thereby facilitate the study of this disease. To date, progress in understanding this infection has been understandably slow because of the lack of a susceptible experimental animal and of a specific test by which to identify the agent of this disease. Should the findings recorded above be confirmed, an SH infection might be diagnosed during its early stages by using the patient's serum as CF antigen with known positive and negative antiserums.

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PROCEEDINGS, TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

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PAPERS

1. Toxicity of copper for *Mycobacterium phlei*. Dr. Darrell Pratt, Department of Bacteriology, The University of Texas, Austin.

2. Antagonism of erythromycin to pantothenate, B-alanine and l-carnosine in growth of *Coryne-bacterium diphtheriae*. Robert G. Brown, Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston.

3. Influence of streptomycin upon population-dynamics during growth of chlorophyll and chlorophyll-less protista. J. Cooke, R. B. Mefferd, Jr., and J. B. Loefer, Southwest Foundation for Research and Education, San Antonio.

4. Germination of *Clostridium* spores in buffered glucose solutions. Donald A. Mehl and E. Staten Wynne, Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

5. Thermophilic germination of spores of mesophilic *Clostridium* species. E. Staten Wynne and Donald A. Mehl, Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

6. Studies on acid-fast organisms in the cockroach *(Periplaneta americana)*, Don W. Micks and Oleta B. McShan, Department of Preventive Medicine and Public Health and Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

7. Environmental control of pigmentation in *Serratia marcescens*. R. B. Mefferd, Jr., and J. B. Loefer, Southwest Foundation for Research and Education, San Antonio.

8. Recent bacteriologic developments in Finland. Etta Macdonald, The University of Texas Medical Branch, Galveston.

9. On the origin of penicillinase-producing resistant Staphylococci. Ara F. Roy and C. E. Lankford, State Department of Health and The University of Texas, Austin.

10. Complement fixation reaction in serum hepatitis. Morris Pollard and Robert Bussell. The University of Texas Medical Branch, Galveston.

11. Antibacterial activity of normal blood and serum. Frank B. Engley, Jr., and Martha Jordan, Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

I. M. LEWIS LECTURE

Dr. Nestor Bohonos

Lederle Laboratories

In vitro Testing of Sensitivity to Antibiotics

12. The stability of stock typhoid vaccine suspensions as determined by total nitrogen determinations and the bacterial count. D. J. Hahn, Mrs. E. B. M. Cooke, and J. H. Arnett, Texas State Department of Health, Austin.

13. Characterization of an antigen for the detection of cutaneous hypersensitivity in brucellosis. Albert A. Benedict and Sanford S. Elberg, Virus Research Laboratory, The University of Texas Medical Branch, Galveston, and Department of Bacteriology, University of California, Berkley.

14. Electron micrographic studies of the yeast cell, S. O. Brown and A. L. Parrack, Department of Biology and Department of Chemistry, A. & M. College, College Station.

15. A comparison of certain ion exchange resins with collodion particles in agglutination. Paul Donaldson and Winona Humes, Department of Bacteriology and Immunology, The Southwestern Medical School of The University of Texas, Dallas.

16. Serum antitrypsin titre in tuberculosis. K. L. Burdon, E. L. Artman, and W. J. Falberg, Department of Bacteriology, Baylor Medical School, Houston.

17. The influence of urethane on the susceptibility of adult mice to coxsackie viruses. Craig Wallis and S. Edward Sulkin, Department of Bacteriology and Immunology, The Southwestern Medical School of The University of Texas, Dallas.

18. Further studies on ornithosis in poultry plant workers. Mrs. Thelma D. Sullivan, J. V. Irons, and M. L. Denley, Texas State Department of Health, Austin.

19. The pathology of an uncharacterized cerebral cortical lesion in laboratory rabbits, with a report on three affected colonies. Melvin Schadewald, Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston.

20. Effect of antibiotics on intestinal clostridia in chicks. J. F. Elam and J. R. Couch. Department of Biochemistry and Nutrition, A. & M. College, College Station.

21. Effect of dietary bacterial cultures on growth of fecal microflora of chicks. G. W. Anderson, Department of Bacteriology, Ontario Agricultural College, Guelph, Canada.

ABSTRACTS

Thermophilic Germination of Spores of Mesophilic *Clostridium* Species. E. Staten Wynne and Donald A. Mehl, Department of Pathology (Division of Experimental Pathology), The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

On incubation at 75° C. in neutral 2 per cent glucose buffered with phosphate, a rapid decrease occurred in countable spores, of *Clostridium botulinum* 62A, *Clostridium botulinum* 115B, *Clostridium chauvei*, *Clostridium perfringens*, and putrefactive anaerobe No. 3679. No significant decrease was observed in Difco brain heart infusion broth with BBL thioglycollate supplement. Slow decreases in spore levels occurred in distilled water, or phosphate buffer, probably due to thermal destruction.

The rapid decrease in countable spores of these mesophilic species following incubation at 75° C. in neutral buffered glucose was considered due to spore germination, since: (1) Addition of 1,000 gamma per ml. of oleate, a known inhibitor of germination, resulted in the drop in spores in buffered glucose becoming essentially equal to that in phosphate alone. (2) Decrease in spore levels was markedly inhibited at pH 5 in 2 of 4 strains tested. (3) Rate of decrease of recoverable spores in putrefactive anaerobe No. 3679 was considerably faster than in *C. botulinum* 62A, in keeping with the previously reported differential in rates of germination in a complex medium at 37° C. (4) Rate of decrease in 2 species tested may be logarithmic, in agreement with previously reported kinetics of germination for these species in a complex medium at 37° C.

A Comparison of Certain Ion Exchange Resins with Collodion Particles in Agglutination. Paul Donaldson and Winona Humes, Department of Bacteriology, Southwestern Medical School of The University of Texas, Dallas.

Agglutination of suspensions of ion exchange resin and collodion particles which had adsorbed antigen coatings from various mixtures of ovalbumin and human gamma globulin were compared after treatment with dilutions of rabbit anti-

human serum. Only collodion particles gave significant agglutination titers when the specific antigen was only one per cent of the total protein present. However, when the gamma globulin amount was over 10 per cent of the total protein, collodion particles were usually nonspecifically agglutinated. The ion exchange resins, Amberlites IR-120, IRA-400 and IRC-50 were less sensitive than collodion particles in that they were not specifically agglutinated when the specific antigen was in small proportion, but they gave significant agglutinative titers following treatment with mixtures of the proteins causing nonspecific agglutination of collodion particles. Suspensions of Amberlite IR-45 were spontaneously agglutinated by ovalbumin but not human albumin. Clear filtrates of mouse carcass suspension caused nonspecific agglutination of Amberlite IR-120, Amberlite IR-45 and collodion particle suspensions.

Environmental Control of Pigmentation in *Serratia Marcescens*. Roy B. Mefferd, Jr., and John B. Loefer. Southwest Foundation for Research and Education, and Trinity University, San Antonio.

A possible influence of streptomycin upon the synthesis of the tripyryllmethene, prodigiosin, comparable to that exerted in some organisms upon the tetrapyryllmethene, chlorophyll, has been investigated. The possibility that the pigment's production and/or maintenance is controlled by cytoplasmic factors (Bunting, *et al.*, 1951, *Amer. Nat.*, 85: 331) which might be inhibited by streptomycin in the manner of chloroplasts required evaluation.

Gradient agar plates (Bunting's medium) containing substances demonstrated to influence pigmentation in *S. marcescens* (lecithin, glutamic acid, sucrose, peptone, phosphate-deficient medium, streptomycin, chloromycetin, neomycin, and anaerobic incubation) were heavily inoculated and incubated at 24 and 37° C. until half maximal surface growth occurred. At that time a complete set of plates from each incubator were cross transferred so that succeeding growth was at the other temperature. Controls were incubated at the original temperature. At 37° C. the lecithin and peptone plates were pink at half maximal growth but all plates were white at maximal

growth. In no case did the 37° C. half-maximal plates develop detectable color upon subsequent incubation at 24° C., although all control plates except those of glutamic acid were pigmented (pink or red) when incubated at 24° C. Although these findings are consistent with the cytoplasmic factor hypothesis, there were important exceptions—often colorless colonies yielded red colonies at 37° C., colorless colonies gave rise to red progeny upon restreaking and incubating at 24° C., etc.

Cultures from standardized inocula (resistant to the levels of streptomycin) were grown in varying concentrations of streptomycin to equal turbidity. Each culture was streaked upon Bunting's agar plates containing different concentrations of streptomycin and the proportion of resulting Red-Pink-White colonies was determined. A progressive decline in the ratio of Red colonies with increasing streptomycin was noted in the control and at low levels (0.05 gamma/ml.), but at higher levels (0.1, 0.5, 0.1, and 2.0 gamma/ml.) the population was stable to the level of resistance in each case. At levels above the resistance of the culture, the decrease was again noted. Although most of these colonies were stable with regards to the pigment as determined by restreaking, several were highly unstable.

Streptomycin seems to exert no direct or specific effect upon prodigiosin. Its effect seems to result from a nonspecific inhibition.

Germination of *Clostridium* Spores in Buffered Glucose Solutions. Donald A. Mehl and E. Staten Wynne, Department of Pathology (Division of Experimental Pathology), The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

Following incubation at 37° C. in neutral 2 per cent glucose buffered with phosphate, a marked decrease occurred in countable spores of *Clostridium botulinum* 62A, *Clostridium botulinum* 115B, *Clostridium chauvei*, *Clostridium perfringens*, and putrefactive anaerobe 3679. Since heating at 75° C. in the germination medium resulted in decreased counts, residual spores were counted after exposure to air to destroy any germinated forms present.

That the decrease in countable spores observed on incubation in neutral buffered glucose was probably due to germination was indicated by the following: (1) No significant decrease in counts occurred in 4 species tested in the presence of 1,000 gamma per ml. of added oleate, a known inhibitor of germination. (2) Counts were practically unaffected at pH 4.8 in 3 organisms studied. (3) Some decrease in countable spores was observed in 3 of 4 species incubated in air, in agreement with previously reported slow germination of these organisms in complex media under relatively aerobic conditions. (4) Rate of decrease of countable spores of putrefactive anaerobe No. 3679 was apparently logarithmic, in keeping with previously reported kinetics of germination of this organism in a complex medium.

Influence of Streptomycin upon Population-Dynamics During Growth of Chlorophyll and Chlorophyll-less Protista. Julian Cooke, Roy B. Mefferd, Jr., and John B. Loefer, Southwest Foundation for Research and Education, and Trinity University, San Antonio.

An analysis of populations of *Euglena gracilis*, *Chlamydomonas agloëformis* and *Chlorella pyrenoidosa* was made to determine different cellular types in normal and streptomycin-treated cultures. In other cases artificial populations of green and colorless strains were established under normal conditions and with streptomycin, and at intervals samples were analyzed with respect to numbers, size of individuals, stages of the life cycle represented and condition of the chloroplasts and chlorophyll. The results demonstrate generally that concentrations as low as 5 gamma/ml. of streptomycin are inhibitory to cellular division, and that in cultures containing this substance, a form resembling a zygote appears very much earlier in the life cycle than in nontreated cultures. In addition, in *Chlamydomonas* cultures, a grossly enlarged morphological type also appears, which sometimes is colorless. Attempts to propagate these morphologically normal colorless forms beyond the second transfer have not been successful, although this limited success and the indirect evidence that too many colorless cells arise in a culture to be accounted for on the basis that they are

all nonviable, leads us to believe that at least some could be propagated on a suitable medium.

While streptomycin does not exert a specific action on chlorophyll or upon green organisms as regards chlorophyll synthesis, its action, depending on the concentration penetrating to the site of action, may be to inhibit cell division, inhibit protoplasmic synthesis, or to interfere indirectly with the chlorophyll picture through affecting the chloroplast. By postulating independent effects of streptomycin acting at various levels on the cell and on the chloroplasts, it is possible to account for the various cellular types observed on a nongenetic basis, although some colorless mutants may occur.

Characterization of An Antigen for the Detection of Cutaneous Hypersensitivity in Brucellosis. Albert A. Benedict, Virus Research Laboratory, The University of Texas Medical Branch, Galveston, and Sanford S. Elberg, Department of Bacteriology, University of California, Berkeley, California.

A skin test antigen capable of detecting hypersensitivity in guinea pigs infected one week was extracted from phenol-killed *Brucella suis* with an anionic surface active agent, sodium lauryl sulfate. The antigen was partially purified and crystallized by precipitation at pH 4–5 and ammonium sulfate precipitation at 6–10 per cent of saturation at pH 7.0 and 5° C. The antigen so isolated was water-soluble near pH 7.0, thermostable and inactivated by trypsin. Tests for the presence of DNA and RNA were negative. Upon purification activity sharply increased when the carbohydrate concentration and the substances responsible for strong ultraviolet absorption at 2,700 Å decreased.

Quantal response data on various preparations were susceptible to analysis of relative potency by virtue of the parallelism of the log dose-log response relationship (Bliss, C. I., *J. Amer. Stat. Assoc.*, 39: 479, 1944).

Repeated intradermal injections of the allergen did not sensitize normal guinea pigs cutaneously or anaphylactically. Low levels of agglutinins (1:8) were induced by repeated intradermal injection of the antigen in guinea pigs.

The detergent-extracted antigen was more sensitive than antigens made of a killed cell suspension, protein nucleate, broth culture filtrate, acid hydrolysate and a polysaccharide, when tested in guinea pigs infected for one week with *Br. suis*.

The use of chromatography and butanol extraction aided in the separation of an agglutinin-stimulating fraction from the skin test antigen. By such a procedure the allergenic fraction was rendered nonantigenic insofar as its ability to induce agglutinin formation following the injection of skin test dose amounts or multiples thereof was concerned.

The Influence of Urethane on Susceptibility of Adult Mice to Coxsackie Viruses. Craig Wallis and S. Edward Sulkin, Department of Bacteriology and Immunology, Southwestern Medical School of The University of Texas, Dallas.

When urethane in drinking water was used as an adjuvant, 15 gram adult mice could be fatally infected with certain strains of coxsackie viruses (Conn.–5 and Nancy). Moreover, serial passage of the Nancy strain could be maintained in adult mice with the aid of this adjuvant. Urethane failed to influence the susceptibility of adult mice to six (6) other strains of coxsackie viruses tested. The report calls attention to several additional features by which certain coxsackie viruses might be differentiated.

The Antibacterial Activity of Normal Blood and Serum. Frank B. Engley, Jr., and Martha Jordan, Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

A survey of animal and human bloods and sera reveals an antibacterial activity apparently unrelated to normal antibody, complement or cellular activity. The activity is present at low dilution levels (1/5–1/20), is heat stable and probably associated with the Beta lysins of the blood.

The antibacterial activity has a wide spectrum. Highest activity was noted against the gram negative microorganisms, especially the coliforms, *Salmonella*, and *Shigella* with the *Proteus* and *Pseudomonas* slightly less susceptible. Of the gram

positive organisms tested, *Bacillus* species were most susceptible, with *Sarcina* less susceptible and *Staphylococcus*, *Diplococcus* and *Streptococcus* even less susceptible.

Neutralization of the antibacterial activity of blood plasma and sera has been observed *in vitro* with complex gums such as acacia, damar and tragacanth, and with other high viscosity suspensions such as gastric mucin. A possible role for gastric mucin in increasing infectivity of organisms *in vivo* is suggested.

The importance or significance of the antibacterial activity suggests itself in routine blood culture technics. Many of the routine procedures add large volumes of blood to culture media resulting in dilutions within the antibacterial range of most blood specimens. Proper dilution of the blood and addition of adjuvants to neutralize the antibacterial activity are proposed.

Antagonism of Erythromycin to Pantothenate, β-alanine and l-carnosine in Growth of *Corynebacterium Diphtheriae*. Robert G. Brown, Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston.

Erythromycin activity against *Corynebacterium diphtheriae* is antagonized by β-alanine or calcium pantothenate in nutrient broth with one per cent tryptose supplement, pH 7, at 37° C. Results of the broth dilution method indicate that β-alanine 500 γ/ml. broth or calcium pantothenate (equivalent to β-alanine, 200 γ/ml.) will allow growth at 70× the original minimal inhibitory concentration of erythromycin (0.02 γ/ml. broth). l-carnosine (equivalent to β-alanine, 100–400 γ per ml.), while effective against low concentrations, is ineffective against a concentration of erythromycin higher than 30× the original minimal inhibitory concentration. No interpretation is made, at present, as to the mode of action of erythromycin versus pathogenic organisms. The suggestions of Frieden (*Tex. Rep. on Biol. and Med.*, 3: 569–646, 1945) may be fruitful in elucidating other mechanisms. Thanks are due Prof. W. B. Sharp for supplying the *gravis* strain used in the present study.

The Pathology of an Uncharacterized Cerebral Lesion in Laboratory Rabbits, With a Report on Three Affected Colonies. Melvin Schadewald,[1] Mental Health Research Laboratory, Hastings, Minnesota.

The chronic toxicity of a number of purified nucleic acids was determined with the eventual prospect of using nucleic acids in therapy and/or prophylaxis against viral infections. Rationale for this work stems from the fact that viruses rapaciously attack the nucleic acids of living tissue in host organisms and it was hoped that supplementation of an abundance of this chemical moiety would replace the vital cellular substrate for the organism until resistance of the host had accumulated to appropriate protective levels. Preliminary work reported by Wright, Bieter, Rossen, *et al.*, had lent credence to this hypothesis. Purified nucleic acids were injected daily into the ear vein of rabbits and the type of response was carefully noted. The nucleic acids thus used were obtained from a number of plant sources as well as from two animal organ sources and one commercially available "Thymonucleic" acid. Neurologic symptoms were not grossly discernible and the young "healthy" rabbits obtained from a commercial supplier appeared to be in every way normal throughout the course of the experiment until they were sacrificed for histologic study.

On histologic examination unmistakable changes were found in the central nervous system. These occurred most frequently in the deeper layers of the cerebral cortex and in the lateral or ventral lateral areas of the mesencephalon. Less frequent lesions were located within the cerebral white matter just outside the internal capsule, within the caudate nucleus, and rarely, but strikingly, in the anterior horn region of the cervical and lumbar spinal cord. In this latter situation the marked resemblance to poliomyelitis was surprising. In several cases not only were the pathological stigmata of an encephalitis visible but the picture was compounded by the presence of a concomitant heavy, chronic leptomeningitis. The lesion varied in its pathological characteristics from a diffuse encephalitis or a focal encephalitis or meningo-encephalitis with a classical

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picture consisting of an early light invasion by amphophils followed quickly by a mononuclear invasion with perivascular cuffing. In three cases definite localized demyelinization was seen in the mesencephalon near the anatomic position of the thalamo-olivary tract. A second type of lesion frequently seen was a chronic granulomatous lesion which showed either a necrotic center of amorphous material with a peripheral broad area of mononuclear invasion and an atypical epithelioid cell formation; or the lesion or lesions consisted of masses of packed "epithelioid" cells with a minimum number of mononuclear invaders. This latter type of lesion superficially resembled a small oligodendroglioma. In all, a total of thirty rabbits exhibited one or several of the variants of lesions just described. And since five untreated animals were examined from this same colony and since these five animals had had no experimental treatment, it was presumed that there was a total affliction without symptoms of the rabbits within this entire lot.

Slides of the lesions were shown to a morphologist working on inflammatory reaction patterns with viruses as well as with allergenic chemical compounds and verbal affirmation was given by this investigator that the lesion which had been presumably produced by nucleic acid injection was indeed present in his experience as a confusing reaction in untreated animals. He had tried four lots of rabbits from different suppliers and in three of these the lesion was "normally" present without experimental manipulation. He indicated that a qualified bacteriologist working with viruses and on serologic reactions had encountered the same confusion in interpretation of the significance of the lesion.

In Texas, opportunity has presented itself to study two apparently normal white laboratory rabbits, one of which showed the lesions in greater abundance than has been heretofore found even in the heavily afflicted colonies at Minnesota. Sections of the inflammatory areas have been cytologically examined but no evidence of virus invasion is thus far discernible. In conclusion, the author cautions investigators using rabbits in experimental work likely to affect the central nervous system without critical evaluation of a misleading "epizootic" neural lesion of surprising and disconcerting proportions.

ELECTRON MICROSCOPIC STUDIES OF THE VIRUS OF VARICELLA (CHICKEN POX) FROM MONKEY SERUM*

REGINALD L. REAGAN, WILLIAM C. DAY, SUZANNE MOORE, AND A. L. BRUECKNER

The incubation period of varicella (chicken pox) is usually 14 to 16 days, with outside limits from 4 to 27 days. Approximately 24 hours after the onset of fever, crops of papules accompanied by vesicles with surrounding erythema appear on the face and trunk, spreading in some cases to the mouth, pharynx, and extremities. The vesicles frequently become pustular and the crusts are often removed by scratching.

Nuclear inclusions have been found in the testicles of monkeys injected with vesicle fluid from varicella lesions (Rivers, 1926). Rake, *et al.* (1948), performed electron microscope studies on vesicle fluid from varicella lesions and found elementary bodies of varicella in these lesions to have an estimated diameter from 210 to 243 mμ. Elementary bodies of varicella virus have been found in vesicle fluids in largest amounts 24 to 48 hours after the appearance of lesions (Rivers, 1948). Experiments by Eckstein (1933) indicated that monkeys could be infected by the virus of varicella. The purpose of the present study was to determine whether the virus of chicken pox could be demonstrated in the serum of infected monkeys by electron microscopy.

Experimental Materials and Methods. Defibrinated venous blood, fluid from vesicle lesions, and nose and throat washings employed in this study were taken from a patient infected with varicella virus. Clinical symptoms of the patient were manifested by the appearance of lesions over the abdomen and face and a temperature of 103.4° F. The infected materials were obtained through the courtesy of Dr. W. L. Dorrance of Children's Hospital, Washington, D.C. These materials were stored at −20° C. for one day before initiating the experiment.

The materials were kept chilled whenever possible, and all glassware was chilled before being used. Monkeys *(Cerco saeus)*

*From the Live Stock Sanitary Service Laboratory, University of Maryland, College Park, Maryland. Accepted for publication October 14, 1952.

weighing one pound were used throughout the experiment. All animals were housed, during the period of the experiment, in individual cages in separate rooms, and isolation precautions were maintained in handling the animals.

The animals were carefully examined each day for the development of changes in the skin, eyes, and inside of mouth. Rectal temperatures were taken each morning. The average temperature of monkeys Nos. 1, 2, and 3 before inoculation with infected material was 99.5° F. The animals were always handled in such a manner as to minimize exertion and excitement before taking temperatures. A diet consisting of milk, bananas, oranges, apples, and raisin bread was provided throughout the experiment.

One monkey, No. 1, was lightly anesthetized with ether and bled from the heart for normal serum. Monkeys No. 1 and No. 2 were exposed intranasally with vesicle fluid and throat washings of above patient, and subcutaneously an dintraperitoneally with blood from the same patient. On the 5th day post inoculation, the rectal temperature of monkey No. 1 reached 102.6° F., and the monkey appeared very nervous. The temperature of monkey No. 2 reached 102.4° F., and occasional vesicles on chest and face were detected. This monkey also had watery eyes, a slight cough, diarrhea, accompanied by some scratching around the area of the vesicles. Each monkey was bled from the heart for 10 ml. of blood. The monkeys survived the bleedings and daily observations of the monkeys were made throughout a 21-day period. The normal serum from monkey No. 1 was kept in the deep freeze and removed on the 5th day at the time that the infected blood was drawn from the heart of infected monkeys Nos. 1 and 2. These serums were then subjected to centrifugation under refrigeration in a Spinco ultracentrifuge for 2 hours at 44,770 r.p.m. The temperature of the refrigerated outer jacket of the centrifuge stayed constant at −10° C. during the centrifugation period. Due to the size of the varicella elementary bodies (210 to 243 mμ) as demonstrated by previous investigators (Rake, *et al.*, 1948), the serums were not filtered. It was believed that filtration would deplete the concentration of the virus to the extent where it would no longer be sufficient for electron study or infective for monkeys. After the 2-hour centrifugation

period, the supernates from the normal and infected specimens were discarded, and the sediment from each specimen was resuspended in 0.5 ml. of chilled physiological saline. Several drops of each suspension were placed on several parlodion film supports which had been prepared 24 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff, 1946) at arc tangent 2/10 and examined under the R.C.A. electron microscope, type E.M.U.

The balance of the concentrated infected serum retained after centrifugation was injected intranasally and subcutaneously into a normal monkey, designated as No. 3. On the 6th day post inoculation, the rectal temperature of monkey No. 3 was 103.8° F. The monkey at this time had diarrhea, a slight cough and occasional vesicles on the abdomen and left foot. After the appearance of clinical symptoms the monkey was observed daily throughout a 21-day period.

Results

Upon electron miscroscope examination of the cencentrated suspension of the serum of the normal monkey before exposure to the virus, no virus-like particles could be seen. Examination by electron microscopy of the concentrated suspension from the infected serum revealed round virus-like particles with a few surface irregularities as shown in figure I. These bodies measured 175 mμ in diameter by direct measurement. These bodies were smaller than the elementary bodies demonstrated in vesicle fluid as described by Rake, *et al.* (1948), and Farrant and O'Connor (1946). No characteristic brick-shaped bodies were found (Rake, *et al.*, 1948). Rounded virus-like particles were the only type structure detected in this study.

Acknowledgments. We are indebted to Col. Edward Palmer of the Medical Service, Walter Reed Army Medical Center, Washington, D.C. for his professional advice offered on the clinical diagnosis of chicken pox.

Summary. Studies by electron microscopy of serums of monkeys infected with the virus of varicella (chicken pox) show the virus to be round in shape with a few surface irregularities. The

virus particles measured 175 mm. in diameter by direct measurement. These bodies could not be demonstrated in normal monkey serum subjected to the same procedure of concentration and electron microscopic examination. They were smaller than the elementary bodies described by Farrant and O'Connor (1946) and Rake, *et al.* (1948).

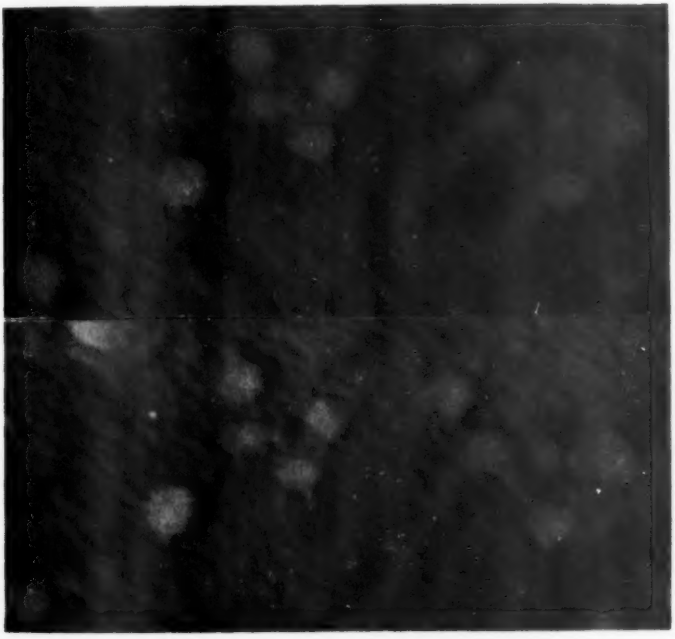

FIG. I. Virus of Varicella. X 60,000.

The virus in the concentrated infected monkey serum produced varicella vesicles on monkey No. 3 on the 6th day post inoculation. On the 6th day this monkey had diarrhea and a slight cough and the rectal temperature was 103.8° F.

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ELECTRON MICROSCOPIC STUDIES OF HERPES SIMPLEX VIRUS AFTER PROPAGATION IN THE SWISS ALBINO MOUSE*

REGINALD L. REAGAN, FRANCES M. SANSONE, SUZANNE MOORE, AND ARTHUR L. BRUECKNER

The virus of herpes simplex (strain HF) used for this study was furnished by the American Type Culture Collection of Washington, D.C. This virus had originally been obtained from Dr. S. Flexner and Dr. P. K. Olitsky. This virus was isolated directly in rabbits which were inoculated intracerebrally with vesicular fluid in 1922 (Flexner and Amoss, 1925; Flexner, 1928). The virus was maintained in rabbits for 11 years by intracerebral passage of infected rabbit brain and was carried through 10 serial passages in mice by intracerebral inoculation in Dr. T. F. McNair Scott's laboratory in the Children's Hospital of Philadelphia. On receipt at this laboratory the virus had been passed 10 times in Swiss albino mice and the infected mouse brains suspended in neutral glycerine.

Material and Methods. The small pieces of mouse brain were removed from the glycerine, ground in a mortar with alundum, and diluted to a 10 per cent suspension with sterile physiological saline. Eleven Swiss albino mice (3-weeks-old) were inoculated intracerebrally with 0.03 ml. of this 10 per cent suspension of herpes simplex virus of the tenth intracerebral mouse passage. The mice showed nervous symptoms of involuntary motor reactions and became moribund on the 3rd day. When symptoms appeared the mice were sacrificed, and the brains were removed aseptically. Eight mice were injected intracerebrally with a 10 per cent suspension of normal mouse brains. No symptoms were noted in these normal controls. These 8 mice were sacrificed on the 3rd day post inoculation. The brains were removed aseptically as above. The pools of infected mouse brains and of normal mouse brains were ground with alundum and diluted to 20 per cent suspensions with physiological saline. The

*From the Maryland State Board of Agriculture, Live Stock Sanitary Service Laboratory, University of Maryland, College Park, Maryland.
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2 suspensions were then subjected to 1-minute centrifugation in an angle centrifuge at 1,000 r.p.m. The supernatants were removed from the specimens and filtered through type ST size L3 Seitz filters. The filtrates were then subjected to centrifugation for 2 hours at 29,880 r.p.m. under refrigeration. The temperature of the refrigerated outer jacket stayed constant at — 8° C. during centrifugation. After the 2-hour centrifugation period, the supernatants from the specimens were discarded and the sediment from each specimen was resuspended in 0.6 ml. of physiological saline. Several drops of each suspension were placed on several parlodion film supports which had been prepared 48 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff, 1945) at arc tangent 2/10 and examined under the RCA electron microscope, type EMU. The balance of the material of each suspension was injected intracerebrally into ten 3-week-old Swiss albino mice.

The mice injected with the concentrated infected material showed nervous symptoms after a period of 2 days. These mice were sacrificed and the brains were removed aseptically, pooled, ground, and diluted to a 10 per cent suspension. This suspension was injected intracerebrally into 10 unvaccinated mice and into 10 mice which previously had been immunized intramuscularly with the tenth mouse passage of herpes simplex virus. After 6 days the unvaccinated mice showed symptoms of central nervous system involvement while the immunized group showed no nervous symptoms. The mice injected intracerebrally with the concentrated suspension of normal mouse brain appeared normal and were discarded after a 6-day observation period. These results confirmed the concentrated virus to be herpes simplex virus.

Results

Upon electron microscope examination of the concentrated suspension of the brains of the normal mice, no virus-like particles could be seen. These controls were screened carefully. Upon examination of the concentrated suspension from the infected brains, uniform virus-like particles were demonstrated

as shown in figure I. These bodies measure 90–100 mμ by direct measurement and are spherical in shape. An irregular contour may be seen on occasional virus particles. There may be 2 particles together giving this impression. Coreill, *et al.* (1950), made electron microscope studies of the elementary bodies of herpes simplex virus (strains HF, EC, and HFE) and found them to measure from 133–233 mμ. in diameter.

FIG. I. Herpes simplex virus shadowed with chromium at arc tangent 2/10. X 20,000.

We are indebted to Ellen Kehne for technical assistance.

Summary. Studies by electron microscopy of brain suspensions of mice infected with herpes simplex (strain HF) show the virus to be spherical in shape with a diameter of 90æ100 mμ. These bodies could not be demonstrated in concentrated suspensions of normal mice brains subjected to the same procedure of concentration and examination. The bodies from the herpes simplex preparation resemble the measles virus described by Reagan, *et al.* (in press). The virus in the concentrated material

was infectious for unvaccinated mice but had no effect on mice previously immunized against herpes simplex virus.

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BIOCHEMICAL AND BIOPHYSICAL METHODS IN CARDIOVASCULAR RESEARCH*

ROBERT F. REDMOND, LAWRENCE J. MILCH, WILLIAM W. CALHOUN, HERMAN I. CHINN, AND THE CARDIOVASCULAR RESEARCH GROUP†

For many years, most of the cardiovascular research was done by the clinical cardiologist in conjunction with his hospital laboratory. Advances within the last decade, however, have emphasized the need for a "team" approach to the problem of heart disease. Consequently, in many laboratories and hospitals, biochemists and biophysicists have combined with clinicians in research teams, so that such organization might take full advantage of the newer concepts that have appeared in the literature.

The Cardiovascular Research Group was formed at the USAF School of Aviation Medicine in mid-1950, and over the past two years certain biochemical and biophysical laboratory procedures were added to existing clinical facilities and subjected to extensive evaluation for accuracy, reproducibility, and adaptability to technical routine. These procedures included:

1. The ultracentrifugal assay of S_f 12–20 lipoproteins.
2. An electrophoretic partition of blood serum proteins.
3. Serum levels of total protein, cholesterol, and phospholipid.
4. Aortal concentrations of cholesterol, phospholipid, and total fat.
5. Calculation of serum colloid osmotic pressure.

Some of the above-listed procedures are adequately described in the literature. The majority, however, although by no means original with us in their fundamental aspects, have not been presented in sufficient detail, and frequent inquiry has been made to this laboratory as to the exact, stepwise, protocols employed. The questions have, in the main, been relevant to the

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ultracentrifugal analyses and the electrophoretic partitions of blood serum. In view of the interest expressed by many physicians and basic scientists, it seems advisable to codify our experiences and publish the results of developmental testing for the benefit of persons unfamiliar with the methods.

All of the above-listed quantitative procedures were calibrated for experimental error. Recovery tests were made in those instances where the pure compound was available. When pure compounds were unobtainable, replicate determinations on pooled serum or tissue samples were performed so as to establish confidence limits.

The purpose of this report, therefore, is twofold. First, to record in technical detail those analytical methods employed in cardiovascular research not already so contained in the literature; and second, to present limits for accuracy, reproducibility, and adaptability to routine.

Ultracentrifugal Analysis

The S_f 12–20 class of serum lipoproteins hereafter referred to as Cholesterol Protein Conjugate (CPC) was first described by Gofman *et al.* (1950), when his group postulated that these cholesterol-bearing protein molecules were associated with atherosclerosis in rabbits and men. The ultracentrifugal estimation of the serum concentration of CPC was devised by the same authors but, to our knowledge, has never been published in detail. The working procedure to be described below is essentially that taught to one of us (L.J.M.) in Gofman's Laboratory at Berkeley, California, in November, 1950. Thus far in the course of this study we have completed almost 1,500 estimations of CPC concentration in the sera of man, rabbit, chicken, and dog, with only slight modifications of the original Gofman protocol. There are, unfortunately, no purified samples of CPC to use for validating this quantitative procedure in an absolute sense. The component, to our knowledge, has not yet been isolated in pure form. However, indirect validation on a relative basis was accomplished and the results of these experiments will be described in subsequent paragraphs.

The quantitative estimation of the blood serum concentration of CPC may be conveniently divided into two relatively inde-

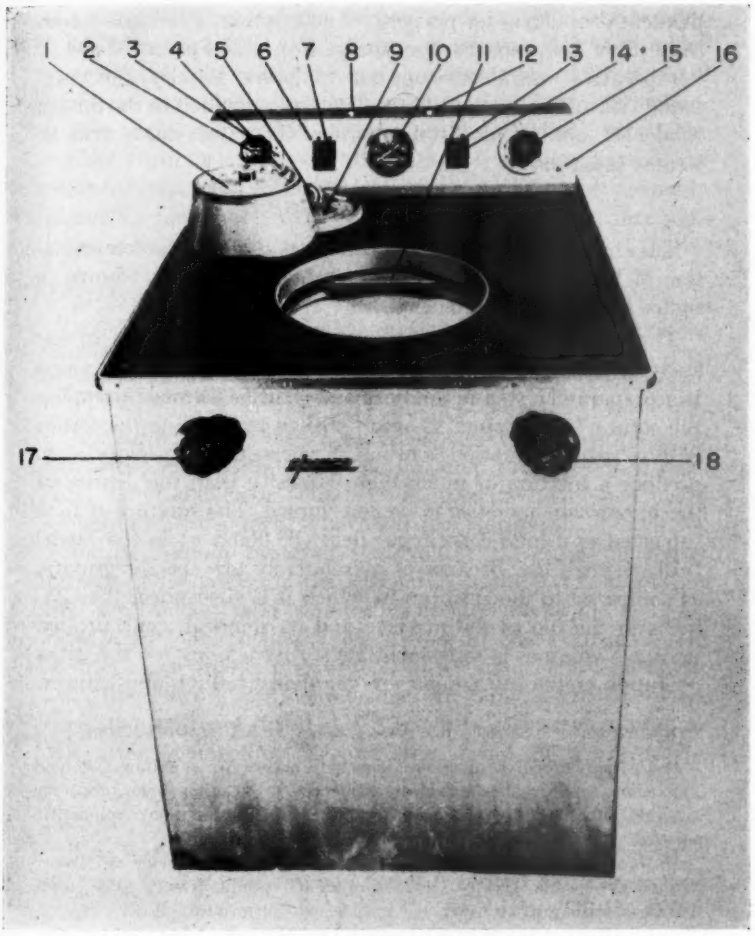


FIG. I. Ultracentrifuge, Model L:

1. Preparatory rotor.
2. Automatic timer.
3. Tube cap.
4. Lusteroid tube.
5. Rotor handle.
6. Refrigerator switch.
7. Power switch.
8. Power pilot light.
9. Rotor cover.
10. Tachometer.
11. Chamber door.
12. Vacuum pilot light.
13. Vacuum switch.
14. Brake switch.
15. Speed control dial.
16. Starting switch.
17. Door control.
18. Vacuum gate control.

pendent procedures for purposes of description. The blood serum must first be prepared for analysis in the Spinco Model L "preparative" ultracentrifuge for 15 hours. Finally, the "prepared" serum is "analyzed" for CPC concentration in the Spinco Model E. analytical ultracentrifuge. The two stages will be treated separately.

Model L Ultracentrifuge

This instrument and component parts used in the determination of the S_f 12–20 lipoproteins of blood serum are shown in figure I, appropriately labeled.

The Model L ultracentrifuge serves the sole function in this assay of concentrating the CPC into a small volume. This process is a preparatory step in analysis and shall be termed "prepping the serum." "Prepping" is accomplished by diluting the serum with a sodium chloride solution of such specific gravity as to produce a mixture of much higher density than the density of the lipoprotein moieties to be determined. The mixture is then subjected to a high centrifugal field (65,000 x g) in the Model L ultracentrifuge. Because of its relatively low specific gravity, as compared to the medium in which it is suspended, the CPC floats to the top of the mixture and its removal, concentrated in small volume, is thus facilitated. Instructions for the gross operation of the instrument are supplied by the manufacturer.

Preparatory Procedure for Assay of S_f 12–20 lipoproteins

Equipment. Model L ultracentrifuge with accessories as follows: 20-hole 30.2 rotor capacity 210 ml., lusteroid tubes to fit rotor holes; caps for lusteroid tubes; cap wrench; 5-cc. volumetric pipettes; 4-cc. volumetric pipettes.

Chemicals and solutions. Aqueous solutions of sodium chloride as follows: solution A—specific gravity 1,132; solution B—specific gravity 1,007; solution C—specific gravity 1,063.

The densities of solutions *A*, *B*, and *C* are checked against appropriately calibrated hygrometers that read directly to four significant figures.

Procedure. The lusteroid tubes are checked for leaks, numbered consecutively with a wax pencil, and placed in a test tube rack of appropriate size. A 5-ml. aliquot of each serum sample is then pipetted into the corresponding lusteroid tube. If there is insufficient serum, an exact amount is pipetted into

the lusteroid tube and the volume made up quantitatively to 5 ml. with salt solution *B*. Four ml. of solution *A* is then added to each tube. The tubes are capped, contents mixed by repeated inversion and placed in the numbered rotor hole corresponding to the wax-penciled number on the lusteroid tube. The rubber vacuum seal in the rotor cover is lightly greased, the rotor cover placed and locked in position and the rotor assembly placed in the ultracentrifuge well. The diluted sera are centrifuged at 30,000 r.p.m. for 15 hours.

The Top Fraction

Equipment. 1-cc. tuberculin syringes; glass-stoppered 1-cc. volumetric test tubes appropriately labeled with the identification number of the sera undergoing analysis; tube-removing tool; cap-removing tool.
Chemicals and solutions. None.

Procedure. After centrifugation has been completed, the rotor is allowed to coast to a stop, the lusteroid tubes removed carefully from the rotor with the appropriate tool and placed sequentially in a rack. The tubes are uncapped and the top 1 ml. of content removed quantitatively with a tuberculin syringe. This portion of the "prepped" serum has been termed the "top fraction" and contains all lipoproteins of the S_f 12–20 class in the 5-ml. portion of original serum. The syringe content is discharged into a glass-stoppered volumetric test tube, the 1-cc. line serving as a double check on the volume. Tubes are then stoppered and stored at 4° C. Solution *C* is used to prepare salt lines (see below, "Quantitation from the Photographic Plate").

Model E Ultracentrifuge

This instrument, with component parts used in the analysis of the prepared top fraction for the serum concentration of S_f 12–20 lipoproteins, is shown in figure II. The Model E ultracentrifuge imposes a high centrifugal field of force on the colloidal particles of lipoprotein suspended in the medium of the top fraction. In addition, however, the Model E machine accomplishes a quantitative analysis of the particle distribution of components by measuring the migration rates of the moving boundaries (see Appendix I and Fig. VII) during ultracentrifugation. Migration rates under a known and controlled gravi-

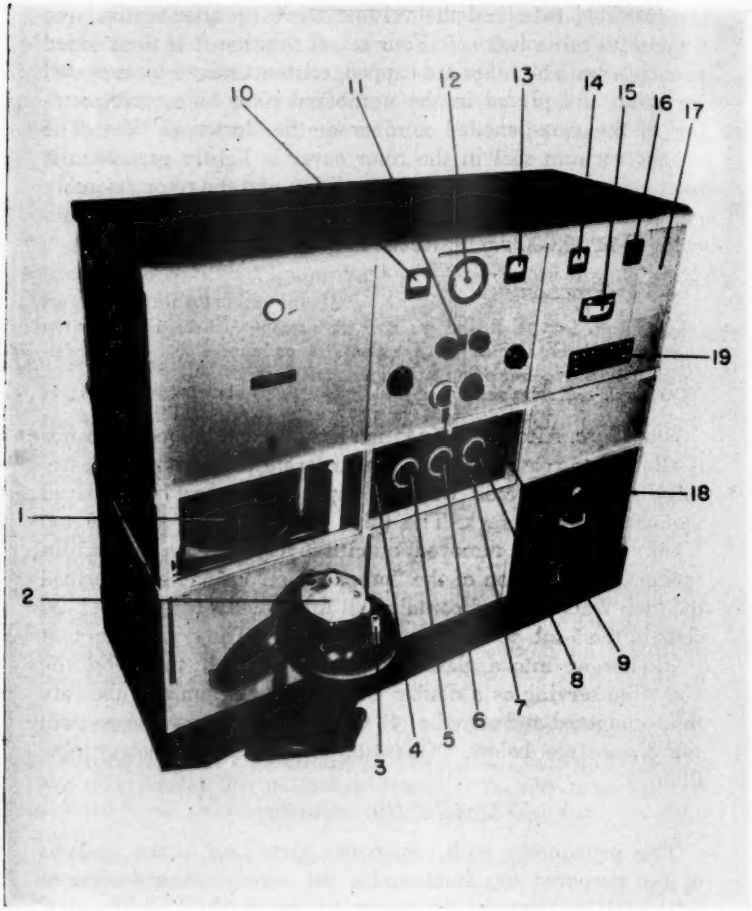


FIG. II. Ultracentrifuge, Model E:

1. Rotor chamber.
2. Analytical rotor.
3. Analytical cell.
4. Vacuum chamber switch.
5. Vacuum pump switch.
6. Diffusion pump switch.
7. Light source voltage switch.
8. Light source switch.
9. Braking switch.
10. Voltage meter.
11. Speed selector.
12. Electric tachometer.
13. Ammeter.
14. Vacuum gage.
15. Thermocouple meter.
16. Viewing screen.
17. Photographic plate compartment.
18. Light source voltage meter.
19. Temperature and camera control panel.

tational force are characteristic of the various components under test, thus identifying them, and the refractive index change they induce in the test mixture is further a measure of their individual concentrations. The instrument automatically records both the rate of migration and the refractive index changes on a photographic plate. Subsequent analysis of the developed photographic plate and appropriate calculations will yield the concentration of S_f 12–20 lipoproteins in blood serum. According to Pickels (personal communication) the concentration is given by a formula:

When.

C=concentration of particular component in starting material in percent.

Θ=angle of analyzing element such as inclined bar to slit image.

L=optical level arm, in cm., distance along optical path from center of condensing lens above rotor to inclined bar.

M=magnification of cell height.

m=magnification of cylindrical lens system.

T=cell thickness in cm.

Δn=specific refractive increment of component (about 0.00154) for lipoproteins in 9 percent salt.

E=magnification used to project photograph for tracing.

N=factor by which component has been concentrated from original to final sample used for analysis.

A=area in cm.^{2} which represents the component.

Ro=distance from axis of rotation in cm. of boundary at start of run.

Ra=distance from axis of rotation of *A* in photograph used for analysis.

Then.

$$C = \left(\frac{A.\tan\Theta}{L.M.m.T.\Delta n.E^2.N}\right) \quad \left(\frac{Ra}{Ro}\right)^2$$

For the machine used in this laboratory if x = mm.^{2} counted.

$T = 1.20$

$Ro = 7.26$

$A = \frac{x}{100}$

$\Delta n = 0.00156$

$Ra = 6.46$

$L = 60.9$

$E = 1$

$\Theta = 45°$

$M = 2.11$

$N = 5$

$m = 3.53$

Procedure for the Assay of Top Fraction

Equipment. Model E ultracentrifuge with modified photographic timer cam installed for S_f 12–20 analysis and with accessories as follows: analytical rotor, 2 holes; 0.35-cc. capacity cells complete and assembled except screw plug and screw gasket out. One cell bears a flat window and the other a "wedged" or prism window; 1-cc. tuberculin syringes with blunted No. 23 needles; the top fraction from two "prepped" serum samples.

Chemicals and solutions. None.

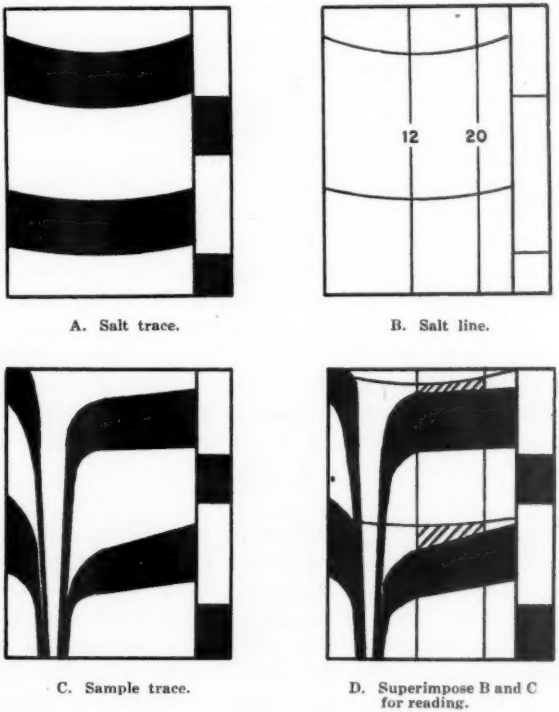
Procedure. A portion of the top fraction from the 1-cc. volumetric test tube is drawn up into the tuberculin syringe and exactly 0.35 ml. discharged into the "flat" cell. The process is repeated with a second "prepped" sample and the "wedge" cell. The cells are fitted into the rotor, and the rotor positioned on the fork and secured to the drive shaft. The free couple temperature is recorded. The chamber is closed and evacuation begun. The light source is checked, photographic plate "run in" and photographic timer set at "hold." The exposure time disc is secured with a clamp in such a position that the first picture will be taken after the time disc has traversed about 40° of revolution. The toggle switch on the shutter mechanism is moved to the automatic photo "off" position.

When the chamber pressure has reached about 5 μ the centrifuge is started and power is maintained smoothly at 14.5 to 15 amperes during the run up. When the speed has reached 40,000 r.p.m. the automatic photo toggle is moved to the "on" position. At 50,000 r.p.m. the exposure disc clamp is removed, and at 52,640 r.p.m. the powerstat is rotated until the voltmeter reads the previously determined maintenance voltage. After 19 minutes of "running," the fixed couple temperature is recorded. After the last picture has been taken, the machine is stopped *with rapid breaking*, the chamber is opened, and the free couple temperature is recorded. The photographic plate is removed and developed.

Modification of Apparatus. It was noted that the resistance across the mercury vapor light source (G.E. AH–6) increased markedly with continued use. The increased resistance under constant current resulted in a corresponding voltage increase. Rough testing indicated that when the voltage load reached 700 volts the light source would fail. It was decided, therefore, to install a voltmeter in series with the light source and replace

the lamp when the voltage approached 700, or after 30 hours (as recommended by Spinco) whichever was sooner. This procedure minimizes the possibility of losing a sample during an analytical run.

Quantitation from the Photographic Plate. Following the development, the plate is analyzed by at least two individuals since, at this point, a certain amount of subjective error is introduced. The analysis is accomplished by comparing the photographic record of salt solution *C* which approximates the final density of the top fraction. Since the salt molecules are so small that they do not migrate, this trace may be used as a base line (see Fig. III). In order to simplify the procedure



A. Salt trace. B. Salt line.

C. Sample trace. D. Superimpose B and C for reading.

FIGURE III

the upper edge of the salt record is drawn onto a sheet of semi-transparent paper and labeled the "salt line." The sample record can now be superimposed on the salt line and the area represented by the difference in position of the two upper edges and confined laterally by the limits of the S_f 12–20 region is a function of the amount of CPC in the solution (see Fig. III). This area is marked out and quantitated in square millimeters. Other workers use various methods of quantitation in square millimeters. Some measure from the middle of the trace; others average values obtained from top and bottom. We have found greater consistency, and thus improved accuracy, by reading from the upper margin.

A number of methods of counting were investigated, including enlargement and measurement with a planimeter and enlargement and counting of squares. These methods were not found to increase significantly the accuracy over counting of squares as viewed through the original plate.

Validation. In order to determine the normal variations inherent in the procedure and method of quantitation, a series of determinations were made on a pool of several samples. A total of ten separate runs were made on sera from this pooled specimen. Each sample was "prepped" individually and separate analytical runs were made on each. For the first run the analytical cell was improperly filled, resulting in a photographic plate which could not be properly analyzed. The last prep run was so altered by bacterial contamination, because of the interval of time that the serum was kept, that it, too, was not suitable. Of the eight remaining determinations, which were analyzed by different pairs of individuals, the values ranged from 34 to 40 mg. per cent. The mean value was 37.2 per cent (see Table 1).

Since, as previously stated, no purified CPC is available for calibration, it was necessary to prove the substance being determined was actually CPC as defined by other workers and to establish the limits of comparison of values from our work with other laboratories. This phase was accomplished by an interchange of data on the same samples with the Belmont Medical Laboratories, Inc., Belmont, California. Blood sera were obtained from 30 individuals and analyzed in our labora-

tory and at the Belmont Laboratories. The Belmont group informed us that, on the basis of previous experience, agreement within a range of 10 mg. per cent would be quite satisfactory. Of 30 samples sent for comparison only 6 were beyond these limits. A second run by our laboratory failed to improve these limits of error. Readings from Belmont showed a tendency to be consistently higher than those made locally. Since 80 per cent of the comparative readings were within the 10 mg. per cent limits previously set up by the Belmont Laboratories, the results of these studies may be considered comparable to those of others in which similar cross-checking was done. An analysis of several hundred samples run routinely by our laboratory in the course of this project shows the mean variation between readings of the same plate to be on the order of 4 mg. per cent.

TABLE 1

Pooled Serum for Reproducibility
(Ultracentrifuge)

Run No.	Result
1	Poor filling
2	34
3	38
4	36
5	38
6	40
7	34
8	38
9	40
10	Bacterial contamination

Range 34 mg. % to 40 mg. %
Mean 37.2 mg. %

The range of values found in approximately 300 males between the ages of 20 and 45 years was from 0 to 192 mg. per cent. The mean value for the same group is 32 mg. per cent.

ELECTROPHORESIS

Introduction. Cohn and his coworkers (1950) have shown that essentially all of the cholesterol of serum is transported as complexes of alpha and beta globulins. In order to study further lipoprotein patterns, the electrophoretic partition of

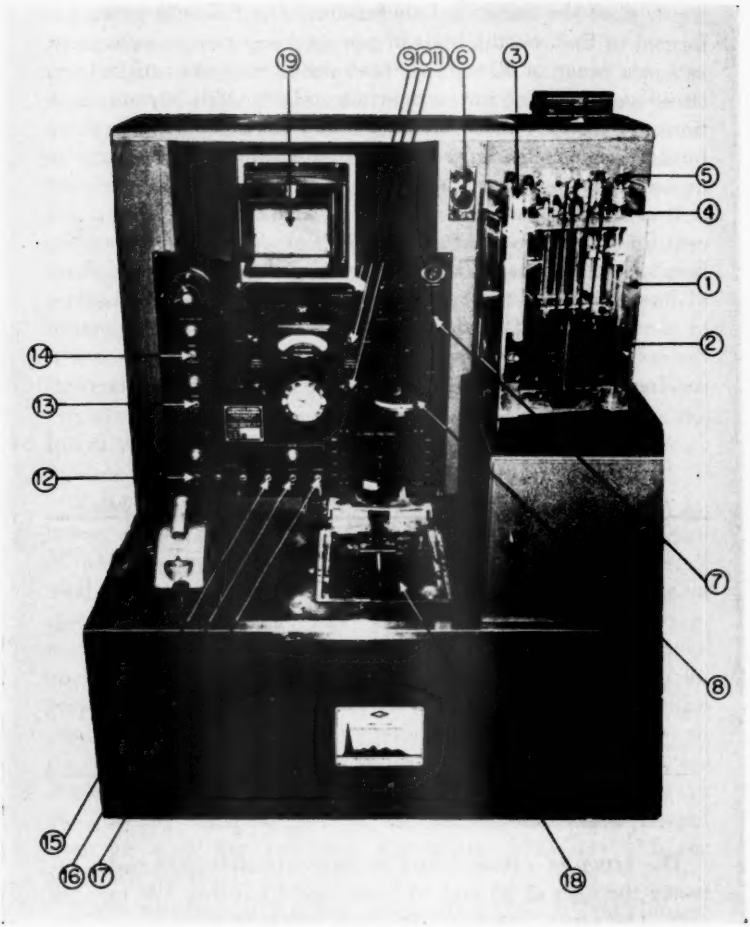


FIG. IV. Electrophoresis apparatus:

1. Cell holder.
2. Shutter.
3. Shutter level.
4. Clinical cell.
5. Cell electrode.
6. Tilt control for mirror.
7. Focusing lever.
8. Diaphragm control.
9. Milliammeter.
10. Coarse current control.
11. Fine current control.
12. Main line switch.
13. Light source switch.
14. High voltage timer switch.
15. Bath light switch.
16. Bath heater switch.
17. Bath stirrer switch.
18. Bath.
19. Screen.

serum proteins was suggested as a method which might yield useful data.

The Aminco-Stern electrophoresis apparatus was utilized for purposes of this partition (see Fig. IV). The technique is a modification of that taught to one of us (L. J. M.) by Miss M. Reiner at Mount Sinai Hospital. The results obtained by the present technique are felt to compare favorably with those of other workers in this field.

The theory of electrophoretic analysis rests on the relative migration rates of differently charged protein particles in a buffered solution under the influence of an electrical field. Moving boundaries[1] are thus established which can then be scanned and integrated by a cylindrical lens system.

In the course of this project, some 750 determinations have been made by the method to be discussed below. General validation of the procedure is well established, but a search of the literature itself has revealed an almost total absence of data regarding reproducibility and the establishment of confidence limits using this apparatus.

Electrophoretic partition requires preparatory treatment of the serum prior to analysis. The preparatory phase involves dilution and dialysis against buffer at near 0° C. The serum is diluted to contain 1.5 gm. per cent protein, transferred to a dialysis bag prepared from cellulose tubing and dialyzed overnight against barbiturate buffer at pH 8.6.

For analysis, the standard clinical cell has been found satisfactory. The filling of the analytical cell is a rather critical procedure requiring skill and dexterity, which are obtained by practice. Technicians selected for this procedure should be capable of rather delicate manipulative activity.

Buffer solution against which the sample was dialyzed is used to fill the cell completely. The electrodes are inserted and an electrolyte composed of salt solution is placed about the electrode. The cell in its carrier is lowered into a constant-temperature alcohol bath maintained at near 0.5° C. and the serum carefully "layered" into the optical portion of the cell. The electrodes are connected to the appropriate terminals and

[1] See Appendix I.

current applied. When resolution of the various boundaries has been obtained, a photographic record is made. Analysis of the photographic film is done by enlargement and comparison of areas planimetrically. Detailed procedure is outlined below. Two specimens may be run simultaneously, which requires duplication of supplies.

Detailed Procedures Preparation of Serum for Fractionation

Equipment and supplies. Approximately 3-ml. serum samples of known protein concentration (see below, "Determination of Total Protein"); barbiturate buffer (approximately 1,000 ml. for each sample); visking cellulose tubing, 1-inch diameter, tested against leaks; 1,000-ml., or larger, beaker for each sample to be dialyzed, and pipettes and glassware for dilution of serum.

Chemicals and solutions. Barbiturate buffer: Diethyl barbituric acid. 18.42 gm.; Sodium diethylbarbiturate, 103.10 gm.; Water q.s. ad 5,000.00 ml.

A suitable piece of visking tubing is moistened in buffer and the potential cavity opened. A firm overhand knot is placed near one end. The tubing is blownup, the free end twisted to close and the membrane tested for leaks by immersion in buffer.

Calculation of dilution for preparing the 1.5 gm. per cent protein solution for analysis is as follows:

$$\text{Ml. serum to use} = \frac{\text{desired conc.} \times \text{desired vol.}}{\text{gm. per cent protein in serum}}$$

Since we desire a 1.5 gm. per cent solution and a total of 12 ml. for our purposes the calculation is reduced to 18/gm. per cent protein in serum. This amount of serum is diluted with buffer, made up to 12 ml. and transferred to the visking tubing. A firm overhand knot is tied in the open end of the tubing and whole immersed into approximately 1 liter of buffer. The beaker is placed in the refrigerated compartment of the machine and allowed to dialyze overnight. The electrical resistance of the buffer and the diluted serum should be equal. This is tested in a conductivity device furnished by Aminco-Stern by means of a conductivity bridge (Model RC 16, manufactured by Industrial Instrument Company). After overnight dialysis, resistance is approximately 4.3 to 4.5 $\times$ 10^3 ohms.

Fractionation of Serum

Equipment and supplies. Standard clinical cell; rack for standard cell (with shuttered mask); dialyzed serum; buffer (used to dialyze serum); conductivity cell (supplied by Aminco); conductivity bridge, Model RC 16 (Industrial Instruments Company); electrodes, silver, coated with silver chloride, attached to hollow stem; burette, 25-ml. capacity, with ground glass stopcock; two pieces of rubber tubing, 3 to 5 cm. x 5 mm. in diameter for connecting burette and syringe to capillary; two electrical leads to electrodes, with banana plug on one end and alligator clamp on the other, and film holders (4″ x 5″) for recording, loaded with film (we have found Contrast process Panchromatic film quite satisfactory).

Solution. Electrolyte solution, 2 M. Either potassium or sodium chloride is satisfactory.

Procedure. The cell is rinsed with a small volume of the buffer used for dialysis. The cell is then completely filled with buffer and placed in the rack. The electrodes are inserted in both electrode wells, taking care that no air bubbles are trapped in any part of the apparatus.

Fifteen ml. of 2 M electrolyte is slowly injected by means of long hypodermic needles through the hollow stem of the electrode shaft, displacing the buffer from about the electrode elements. The mask is then attached to the rack and the entire assembly lowered into the alcohol bath.

The specimen to be analyzed is removed from the visking tubing by decanting into a burette equipped with a ground glass stopcock. The burette tip is attached to the entrance capillary with a short piece of rubber tubing. A hypodermic syringe is attached to the side arm of the entrance capillary and, by suitable manipulation, all air is removed from the system before the serum is allowed to run in.

After removing the cylindrical lens from its holder in the back of the machine the progress of the serum may be observed on the ground glass screen by allowing the scanning light beam to pass through the optical portion of the cell. When the serum has progressed to a point approximately 3.5 cm. from the edge of the screen on the ascending limb, filling is stopped. If sufficient care has been taken and the flow of diluted serum is sufficiently slow, then a very sharp boundary will be visible. If the boundary is not sharp, filling is unsatisfactory (see Fig. V-A).

The cylindrical lens is now checked for cleanliness and carefully replaced in its holder. The lens is rotated slightly until the base line is horizontal. A single sharp peak should now appear at the position of the boundary (see Fig. V-B).

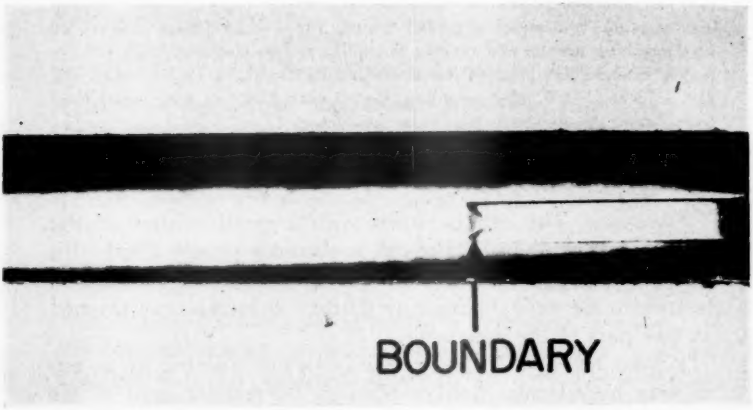


FIG. V-A. Appearance of screen without cylindrical lens.

The cell is now ready for the application of an electrical field. The electrodes are connected in such a manner that the positive lead is attached to the ascending limb terminal. Field strength is adjusted so approximately 5 to 7 V/cm.^{2} is applied (for serum samples herein described, 15 ma. and $\simeq$ 4,600 ohms). After a period of about 80 to 110 minutes the peaks are well-defined and a photographic record is made. Some judgment must be exercised in choosing the optimum time of recording. If the film is exposed too early, resolution is incomplete; on the other hand, if excessive time has passed, the boundaries are so flattened that accurate delineation of components is difficult (examples of satisfactory resolution are seen in Fig. VI).

After development of the photographic record the film is enlarged to fit suitably on an 8½ x 11-inch sheet of paper and the upper edge of the curve is carefully traced on the paper. The low points dividing the peaks are carefully marked and the base line established. The areas representing the various

components are quantitated by means of a Keuffel and Esser planimeter.

Modifications of Apparatus. Early in our experience with Aminco-Stern electrophoresis apparatus, two alterations of the original equipment were made. Both modifications are simple, but each was found to materially increase the efficiency of operation.

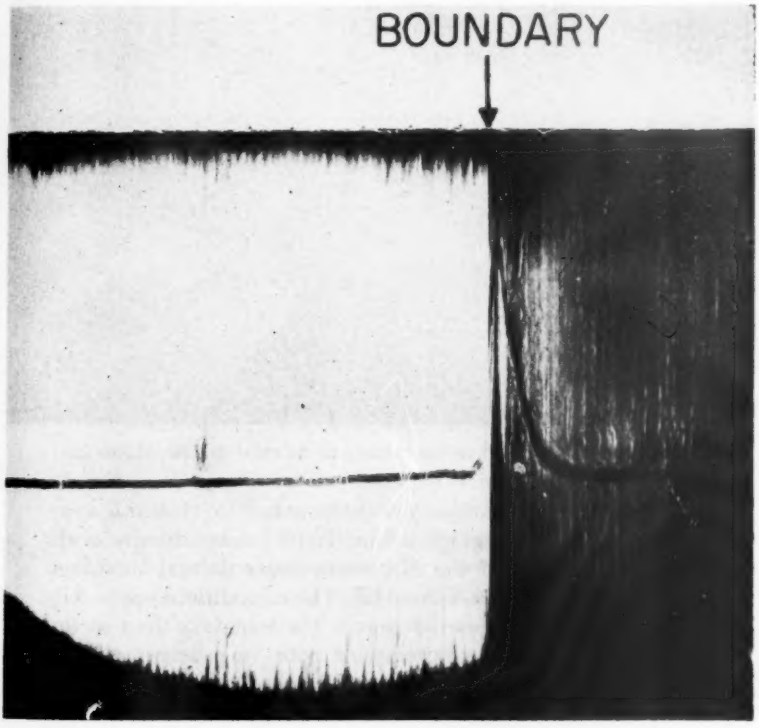


FIG. V-B. Appearance of screen after proper filling of standard cell; cylindrical lens in place.

The first modification resulted from difficulties arising in accurately positioning the shutter mask prior to placing the rack in the well. The simple lever adjustment designed in this

laboratory is shown in figure IV. This device allows adjustment of the shutter mask at any time during the operation of the apparatus.

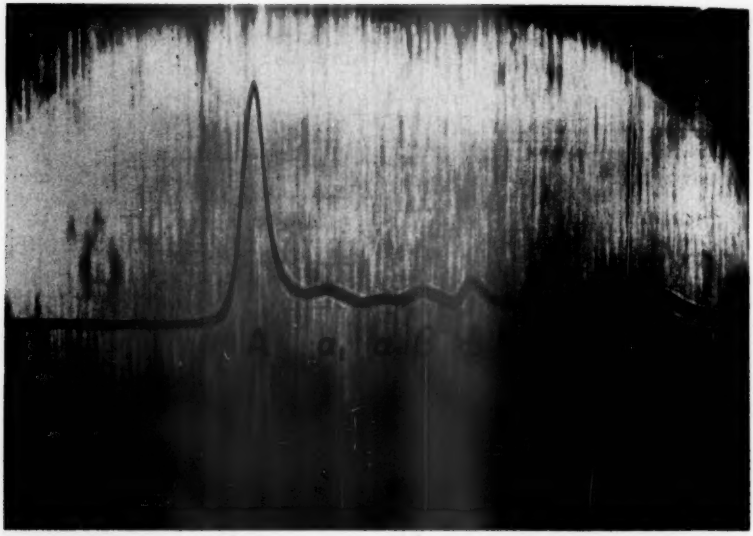


FIG. VI. Example of an electrophoretic diagram (rabbit plasma).

A second complication was encountered in obtaining even exposure of the photographic film. The increased density of the right-hand portion of the film even under normal conditions may be seen in figures V-A and B. These conditions apply only to the ascending portion but this is the boundary used in this study. Under abnormal conditions with very dense solutions the film exposure latitude was so markedly exceeded that it was impossible to follow the trace throughout its course. The obvious answer was to increase the exposure of the denser portion, but this would have been technically difficult since the slide in the film holder was operated from the right on the original equipment. This was obviated by the simple expedient of reversing the plate holder so that the slide could be moved only far enough to expose the denser portion, then completely

removed to give the remainder of the film only that amount of exposure required by its lesser density.

Validation. In an effort to establish the limits of error in the procedure, a pool of rabbit serum was divided into seven aliquots and a separate electrophoretic analysis was made on each portion. These values, with the exception of the albumin/globulin ratio, are given in per cent of total serum protein. The results of these replicate analyses are shown in table 2.

TABLE 2

Pooled Rabbit Serum
Reproducibility, Electrophoresis

Aliquot	A/G	%A	$\%_1$	$\%_2$	%β	%γ
A	1.27	56	7	10	15	12
B	1.38	58	7	10	15	10
C	1.08	58	7	13	15	13
D	1.04	51	6	12	17	14
E	1.09	52	7	12	17	12
F	1.28	56	8	11	13	11
G	1.76	64	6	8	12	11
S.D.	0.25	4.6	0.70	1.7	1.9	1.3

DETERMINATION OF TOTAL PROTEIN

Determination of total protein in the course of this investigation was done by use of a quantitative biuret reaction, as described by Gornall, Bardawill, and David (1949). The procedure has been well evaluated by many individuals, including the above-mentioned authors. This method is relatively simple and quick, yielding quite satisfactory results.

In our initial series on flying personnel, the mean value for serum total protein was 7.33 gm. per cent. The range for this presumably normal group is from 4.48 to 9.35 gm. per cent.

DETERMINATION OF SERUM CHOLESTEROL

A number of methods for the determination of total serum cholesterol have been proposed. Of these, three were tried in this laboratory before choosing the method of Bloor (1916) as recorded in Hepler's manual (1950). The Schoenheimer-Sperry technique (1934) was not found to be superior to the present

method for total cholesterol, and the Fister (1950) modification of the method of Sobel and Mayer (1945) was not suitable in our hands because of difficulties with reproducibility. Careful control of time-temperature relationships was found necessary for accurate results.

Validation of Cholesterol Method. The entire series of determinations of cholesterol utilizing the various methods will not be discussed in this paper. Only that group of data obtained in the evaluation of the Bloor-Hepler technique will be presented.

The results of multiple runs on a pool of human serum are recorded in table 3. These values show a remarkably good reproducibility, with variations not exceeding 3 per cent in twelve separate runs on six extracts of serum.

TABLE 3

Duplicate Determinations of Serum Cholesterol from Each of Six Separate Extractions of Aliquots of a Pool of Human Serum

Extract	Duplicate	Result in mg. percent
A	1	200
	2	198
B	1	200
	2	195
C	1	200
	2	198
D	1	198
	2	195
E	1	198
	2	198
F	1	200
	2	203

The results of recovery experiments are shown in table 4. Six aliquots were taken from a pool of human serum. Cholesterol was added to five of them by increments of 50 mg. per cent equivalents. The results show a range of variation from

less than 1 per cent to 5.6 per cent, with an average variation of less than 2 per cent. Duplicate determinations made from time to time during the series show a similar range of variability.

TABLE 4

Recovery Experiments
Bloor's Cholesterol Method

Sample	Expected	Result	Percent
Normal serum	...	207	.
Serum + 50 mg.	257	255	< 1
Serum + 100 mg.	307	290	5.6
Serum + 150 mg.	357	364	2
Serum + 200 mg.	407	401	1.3
Serum + 250 mg.	457	454	< 1

In over 300 determinations of total serum cholesterol made on flying personnel in the early phases of this study, the mean value was 211 mg. per cent, with a range of 137 to 352 mg. per cent.

Determination of Serum Phospholipid

Phospholipid determinations on blood sera were carried out by modification of a method of Youngsburg and Youngsburg (1930) which utilizes the method of Fiske and Subbarow (1925). The determination is moderately difficult and considerable care is required to achieve reproducibility. Losses may be encountered during any of the multiple transfers or during digestion.

Phospholipid determinations were carried out in duplicate until the workers were sufficiently skilled to warrant dispensing with this additional burden. Duplicate determinations were made on at least one specimen in each daily series. The range of difference between duplicates was 0 to 7 per cent, with mean variance of 2.5 per cent. Human values range from 170 to 434 mg. per cent. The mean value by this method is 278 mg. per cent in a series of almost 300 individuals.

Determination of Aortal Cholesterol

The removal of cholesterol from pulverized dried aortal tissue was accomplished by means of a continuous extraction with a

chloroform-alcohol mixture. Cold finger condensers, similar to those used by Hsaio (1949), were fitted into small micro Kjeldahl digestion flasks. The cold fingers were equipped with a ring from which a fat-free extraction thimble was suspended. A weighed amount of dry aortal tissue was placed in the thimble and the thimble then suspended from the ring on the cold finger so as to hang above the surface of the solvent mixture. With water flowing through the condensers the entire assembly was placed in a heating mantle and warmed until a steady drip of the chloroform-alcohol mixture passed through the content of the thimble. Extraction was allowed to continue for two hours.

Experiments were carried out to determine the length of time and the best solvent required for complete extraction of cholesterol from aorta by this method.

Procedure. Samples of aortal tissue are dried to constant weight in a 60° C. oven and finely pulverized. A weighed amount of tissue is placed into a fat-free extraction thimble and extracted for two hours with a mixture of three parts chloroform and one part alcohol. The extract is transferred quantitatively with washing to a small beaker and evaporated to dryness. This quantity is again transferred with washing to a 10-ml. volumetric flask and made up to a volume of 10 ml. with chloroform. The entire amount, representing all of the extracted cholesterol, is used for color development with the addition of acetic anhydride and sulfuric acid as for serum cholesterol.

Several solvents were tried. The best results were consistently obtained with a 3:1 chloroform-alcohol mixture as described above. Chloroform alone, alcohol alone, and a 1:1 mixture of chloroform-alcohol were found to be inferior. The additional time and effort involved in following a first solvent by various other solvents or combinations was not found to significantly increase the amount of cholesterol extracted.

Additional studies were made to determine the optimum time required for extraction. Some of the results of both studies are shown in table 5.

Extraction for periods longer than two hours failed to show significant increase in cholesterol extraction. If cholesterol de-

termination alone is desired, then chloroform-alcohol 3:1 is a satisfactory solvent. If both cholesterol and phospholipid are to be determined in the same sample, a multiple solvent technique may be desirable (see section below on aortal phospholipids).

TABLE 5

Extraction of Cholesterol from a Pool of Dried Beef Aorta

Solvent	Mg./gm. dry tissue extracted in 1 hr. Range	Mean	Mg./gm. dry tissue extracted in 2 hr. Range	Mean
Alcohol alone	2.94–3.02	2.88	....	...
Chloroform alone	3.32–3.70	3.53	3.38–3.69	3.54
Chloroform-alcohol 1:1	2.94–3.46	3.22	3.78–3.93	3.89
Chloroform-alcohol 3:1	....	...	4.15–4.60	4.39
Chloroform-alcohol 1:1–2 hr. followed by chloroform-alcohol 3:1–1 hr.	....	...	4.46–4.54	4.5

DETERMINATION OF AORTAL PHOSPHOLIPID

Extraction of phospholipid from aortal tissue is carried out in the same type apparatus described above under "Determination of Aortal Cholesterol." All extractions are done for two hours on desiccated aortal tissue prepared as for cholesterol extraction.

Extraction is accomplished with a mixture of equal parts of alcohol and chloroform. Alcohol and chloroform were tried separately, but extraction was incomplete. The various methods tried and the discussion of the method are presented under "Procedure."

Procedure. Tissue samples prepared as for the determination of aortal cholesterol are extracted for two hours in a chloroform-alcohol, 1:1, mixture. The total extract is transferred with washing to a digestion tube and evaporated to dryness. The residue is taken up in alcohol-ether, 3:1, mixture and again evaporated to dryness. At this point 5N sulfuric acid is added, as in the procedure described for the serum determination. Digestion is allowed to progress until thorough charring is observed and allowed to continue for ten minutes thereafter.

Thirty per cent hydrogen peroxide (Superoxol) is then added. From this point the procedure parallels the serum determination.

A number of solvents and varying times of extraction were tried in an effort to obtain optimum conditions. The two-hour 1:1 chloroform-alcohol combination was found to be the method of choice.

Of some interest to others working in this field was the observation that a 1:1 chloroform-alcohol mixture followed by a 3:1 corresponding mixture yields higher phospholipid values than extraction in the reverse order. This procedure is of value when efforts are made to determine both cholesterol and phospholipid on the same sample.

The results of the time-solvent studies are shown in table 6.

TABLE 6

Extraction of Phospholipid fro ma Pool of Dried Beef Aorta

Solvent	Mg./gm. dry tissue extracted in 1 hr. Range	Mean	Mg./gm. dry tissue extracted in 2 hr. Range	Mean
Chloroform alone	5.25–6.00	5.63	5.75– 6.00	5.88
Alcohol alone	7.25–8.00	7.63	10.75–11.50	11.13
Chloroform-alcohol 3:1	....	...	9.93–11.33	10.63
Chloroform-alcohol 1:1	....	...	16.4 –18.9	18.2
Chloroform-alcohol 1:1–2 hr. followed by chloroform-alcohol 3:1–1 hr.	....	...	18.4 –19.0	18.7

As stated above, a 1:1 chloroform-alcohol mixture followed by a 3:1 corresponding mixture yields higher cholesterol values than extraction in the reverse order. If only phospholipid is to be determined, satisfactory results may be obtained from a 1:1 solvent. If both cholesterol and phospholipid determinations are desired, then 1:1 followed by 3:1 allows both determinations to be carried out on a single aliquot of dry tissue.

DETERMINATION OF TOTAL FAT OF AORTA

The total aortal fat content was determined gravimetrically by careful weighing before and after extraction for cholesterol

and phospholipid. Total aortal fat was found to roughly parallel the cholesterol content.

Calculation of Serum Colloid Osmotic Pressure

Serum colloidal osmotic pressure was calculated by the formula of Meyer (1951).

$$P = \frac{\sqrt{q + 0.2 + 2.185}}{\frac{1}{p} + 0.0182\,(q - 1.39)^2 - 0.0415}$$

P = colloid osmotic pressure (atmospheres).
When p = protein concentration in gm. per cent.
Q = albumin/globulin ratio.

This value in humans is found to vary from 19.9073 to 53.3877 in a series of 205 calculations. The mean value for this group was 34.7641.

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Appendix I

The principle of the moving boundary as applied to biophysical measurements may be illustrated by an analogy.

If particles of sufficient density to settle out on standing are suspended in a liquid medium, *e.g.*, silt in water, and are not agitated for a period of time the heavier particles begin to fall through the solution under the influence of gravity. If all particles are of equal density the rate of sedimentation is constant and shortly a clear zone appears in the upper portion of the vessel (Fig. VII). As the time interval increases, the clear zone widens.

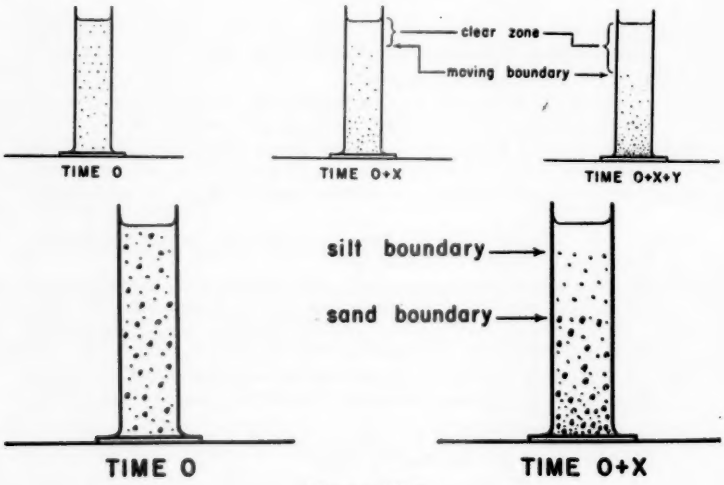

Figure VII

The development of a moving boundary in a monodisperse system as represented by silt settling in water leaving a clear zone.

The development of moving boundaries in a polydisperse system; the heavier sand particles settle more rapidly than silt.

The time required to clear a certain distance depends on the rate of sedimentation which, in turn, depends on the difference in density between the silt and water. This simple silt-water suspension represents a monodisperse system. The addition of other components of greater or lesser density forms a polydisperse system which results in an additional moving boundary for each sedimenting component. If there is a gradation in density, then there will be no definite boundary, but a gradual increase in the density of the system from top to bottom.

The number of particles at the boundary represents the number of particles originally present in the same volume of the original solution as is represented by the volume of the boundary. The above statement is valid so long as other forces such as friction, convection, diffusion, etc., are controlled and until the concentration is increased by piling up against the bottom of the container.

If the boundaries are scanned by a beam of light, changes in refractive index will be observed at each boundary. The amount of change in refractive index depends on the number of particles suspended in the solution at that level. The refractive index gradient is recorded on a photographic plate after optical integration by a rather complex cylindrical lens system. A complete description of this lens system is available in the literature (Philpot, 1938; Anderson, 1939; Longworth, 1946).

In the determination of lipoproteins, flotation instead of sedimentation is employed, not only in concentrating the lipoproteins from other high-molecular-weight components, but also in analysis. The moving boundary principle, in this case, operates against gravity by virtue of a negative density difference, as compared to the illustrative analogy given above.

For convenience, the various components of the system analyzed are identified by the sedimentation constant—usually given in "S" or Svedberg units. The mathematical derivation and explanation of this unit is available from several sources in the literature (Pickels, 1950; Svedberg, 1940; Nickols, 1949). Since the lipoproteins considered in this work float, they are designated "S_f" or *Svedbergs of flotation.*

ACUTE ANEMIA PRODUCED BY PHENYLHYDRAZINE HYDROCHLORIDE IN THE DUCK: OBSERVATIONS ON NUCLEATED ERYTHROCYTES *IN VIVO*•

R. H. Rigdon

Observations on the effect of phenylhydrazine hydrochloride on nonnucleated erythrocytes have been made by Rigdon, *et al.* (1950), and Rigdon and Breslin (1951). It was observed that the monkey's erythrocytes were affected within a few hours following an oral dose of this drug. The hemoglobin within the cells became granular and some of the cells progressively increased in size until they ruptured (Rigdon, Micks, and Breslin, 1950). The theories relative to the pathogenesis of these degenerative changes in the nonnucleated erythrocytes and the formation of the so-called Heinz-Ehrlich bodies were discussed in a recent paper by Webster in 1949.

Few observations have been made on the effect of hemolytic agents on nucleated erythrocytes *in vivo*. Heinz in 1890 observed a difference in the effect of phenylhydrazine on nucleated and nonnucleated red cells *in vitro*. Granules appeared in the hemoglobin of the former type of cell, while nucleated red cells from the frog showed a swelling of the nucleus with loss of its oval shape. No granules were present in the cytoplasm. Bordet in 1899 observed the effect of rabbit serum on chicken erythrocytes *in vitro*. In this experiment the red cells agglutinated and nothing remained of the protoplasm but a very delicate stroma. The nuclei stained normally. In 1937 Weinman concluded from a review of the literature that hemolytic sera in the experience of most workers do not affect the nuclei. However, he described a natural hemolysin in rats that did produce degenerative changes in both the cytoplasm and the nucleus of chicken erythrocytes. Nuclei of pigeon red cells normally stain blue with either Wright or Proca stain; when hemolyzed the nuclear remnants stain pink (Gay and Clark, 1934). In 1902, Stewart observed the behavior of nucleated red

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cells to certain hemolytic agents *in vitro*. He noted difference between the nuclear and the extranuclear portion of the intact corpuscle. He described the process of crystallization of the hemoglobin in erythrocytes from *Necturus* treated with hemolytic agents. It is of interest to note from these *in vitro* observations that the cytoplasm and nucleus of cells from different hosts may react differently in the presence of the same hemolysin.

In 1928, Shattuck concluded that the kinetics of hemolysis on nucleated red cells, as produced by the simple hemolysins saponin, sodium taurocholate and sodium oleate, were unknown. In this study of the action of these agents on chicken erythrocytes, he observed, "After the lysin is added to the cells, it gradually loses its pointed ends and its ellipsoidal shape and becomes circular in flat view. After assuming this spherical shape, it gradually fades from view leaving what appears to be a smaller nucleus in the center of a spherical ghostcell." Sabin in 1921 said, "The criterion I have used for the actual death of the cell is whether the nucleus stains or not."

Our experimental observations of the effect of phenylhydrazine hydrochloride on the duck's erythrocytes *in vivo* are reported.

Methods and Materials. White Pekin ducks from two to ten weeks of age were used. Blood for the erythrocyte counts was obtained from the web of the foot. Hayem's diluting fluid was used. Smears were stained with a combination of Wright and Giemsa stain, while some were stained only with Wright. Standard techniques were used in counting the erythrocytes.

A solution of phenylhydrazine hydrochloride was prepared by dissolving one gram of the crystals in 50 milliliters of 95 per cent ethyl alcohol. This solution was put into the stomach through a small catheter. Blood smears and erythrocyte counts were made at frequent intervals for several days after this drug was given. Since the blood became alkaline in reaction, it was necessary to buffer the stains with a solution of sodium phosphate of pH 6.6.

Erythroblasts were recognized by the round shape of both the cell and the nucleus and by the bluish-staining of the cytoplasm. The number of erythroblasts counted in a Wright-

Giemsa stain usually was less than when counted in a specific reticulum stain. The number of young red cells and injured erythrocytes within the circulation was counted per 500 erythrocytes.

Supravital staining techniques were used to observe the morphologic changes in the erythrocytes. A one per cent solution of brilliant cresyl blue in physiological saline was used. One drop of this stain was put on a glass slide and a drop of fresh blood was put on a cover slip. The latter was placed immediately over the former. After a few minutes at room temperature the erythroblasts were counted per 1,000 red blood cells.

Fifteen ducks given phenylhydrazine hydrochloride were killed at intervals from 6 hours to 14 days and their tissues were studied. Histologic sections were prepared from the liver and spleen. They were stained routinely with hematoxylin and phloxine and by the potassium ferrocyanide technique for iron. Ten normal ducks were used as controls for these histological observations.

Smears were made from the bone marrow of five ducks given phenylhydrazine hydrochloride. These were stained with Wright's stain and a differential count was made on 500 cells from each bird.

Experimental. The number of erythrocytes in the peripheral blood of two normal ducks was determined over a period of ten days. These data are shown in figure I. The number of red cells varied normally between 1.9 and 2.5 million per cubic millimeter and the number of erythroblasts was less than one per 500 erythrocytes when counted in Wright-Giemsa stained smears. Nine ducks used for hematological study were given one dose of the phenylhydrazine hydrochloride solution; one milligram of phenylhydrazine was given per 21.5 to 37.0 grams of body weight. The effect of this hemalytic agent on the total number of erythrocytes is shown in figure I. It is obvious that the number of red blood cells rapidly decreased following the oral administration of phenylhydrazine hydrochloride. This diminution started within five hours and persisted for approximately 48 hours. Four days after this drug was given, the erythrocyte count had returned to approximate normal levels (Fig. I). Erythroblasts appeared in the peripheral

blood approximately six hours following the time of administration of the drug. They rapidly increased in number as the total number of erythrocytes decreased. Then the number of erythroblasts rapidly decreased with the return of the erythrocytes to normal levels.

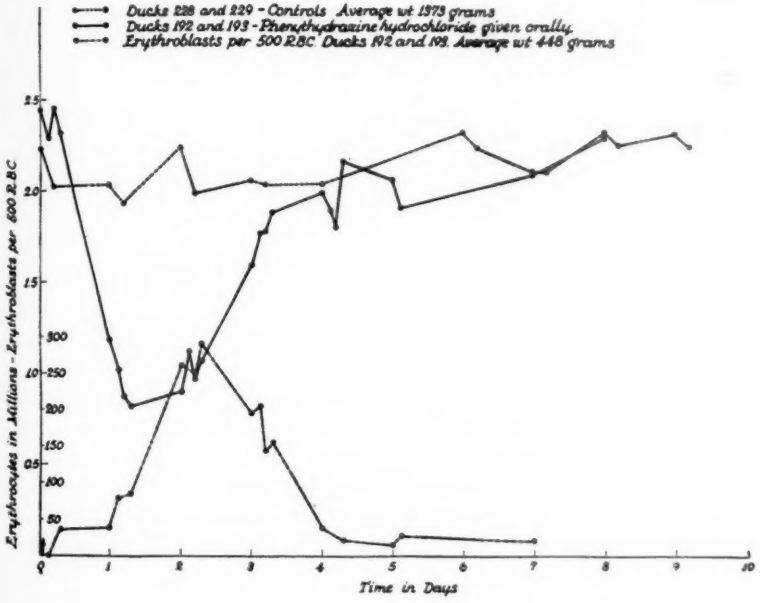
Ducks 228 and 229 - Controls Average wt 1975 grams
Ducks 192 and 193 - Phenylhydrazine hydrochloride given orally.
Erythroblasts per 500 R.B.C. Ducks 192 and 193. Average wt 448 grams
Erythrocytes in Millions - Erythroblasts per 500 R.B.C.
Time in Days

FIG. I. Effect of phenylhydrazine hydrochloride on the total number of erythrocytes in the peripheral blood of the duck. Note the increase in number of erythroblasts accompanying the development of the anemia and their decrease when the erythrocyte count returns to normal. 20 Mg. of phenylhydrazine hydrochloride were given orally.

Figure II shows the effect of a second dose of phenylhydrazine hydrochloride on the erythrocytic and erythroblastic counts. The decrease in the number of red cells at this time indicates that phenylhydrazine hydrochloride is injurious to erythroblasts. Our data are insufficient, however, to determine whether erythrocytes or erythroblasts are more susceptible to the action of this drug.

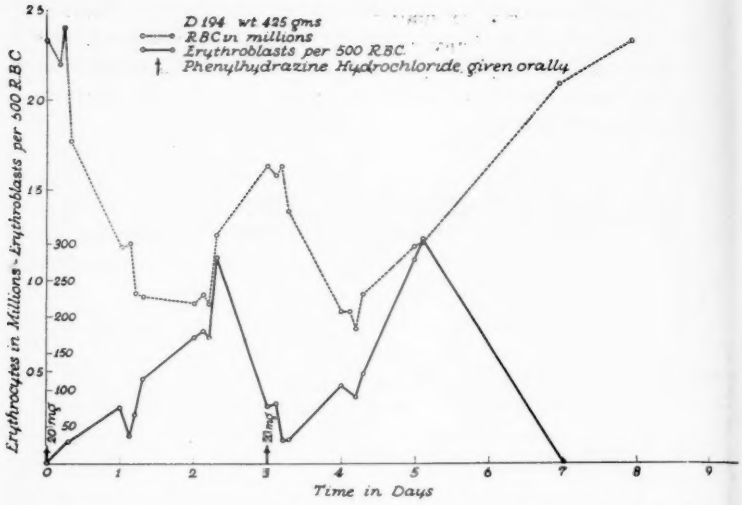

FIG. II. The number of erythrocytes and erythroblasts decrease in the circulating blood following the oral administration of phenylhydrazine.

It is obvious from the above observations that nucleated red blood cells are destroyed in the peripheral blood by phenylhydrazine hydrochloride. The most conspicuous morphologic change occurred in the nucleus of the injured erythrocytes; in fact, few specific changes were observed within the cytoplasm of these red cells. The first variation to be observed was in the staining of the nuclei. Normally they stained purple (Fig. III-A), but following injury they stained pink (Fig. III-B and D). Subsequently the nucleus and cytoplasm became larger and the cell membrane apparently ruptured. The hemoglobin stained progressively lighter until only a faint trace could be seen (Fig. III-D). In contrast to the light staining of the cytoplasm, the nuclei increased in size, stained deeply pink and fragmented before they disappeared from the peripheral blood. The average number of injured erythrocytes in the circulating blood of four ducks given one dose of phenylhydrazine hydrochloride was as follows: after three hours, 2.5 cells per 500 erythrocytes; after five hours, 5.5 cells; twenty-four hours, 14.25 cells; twenty-seven hours, 12.0 cells; thirty-two hours,

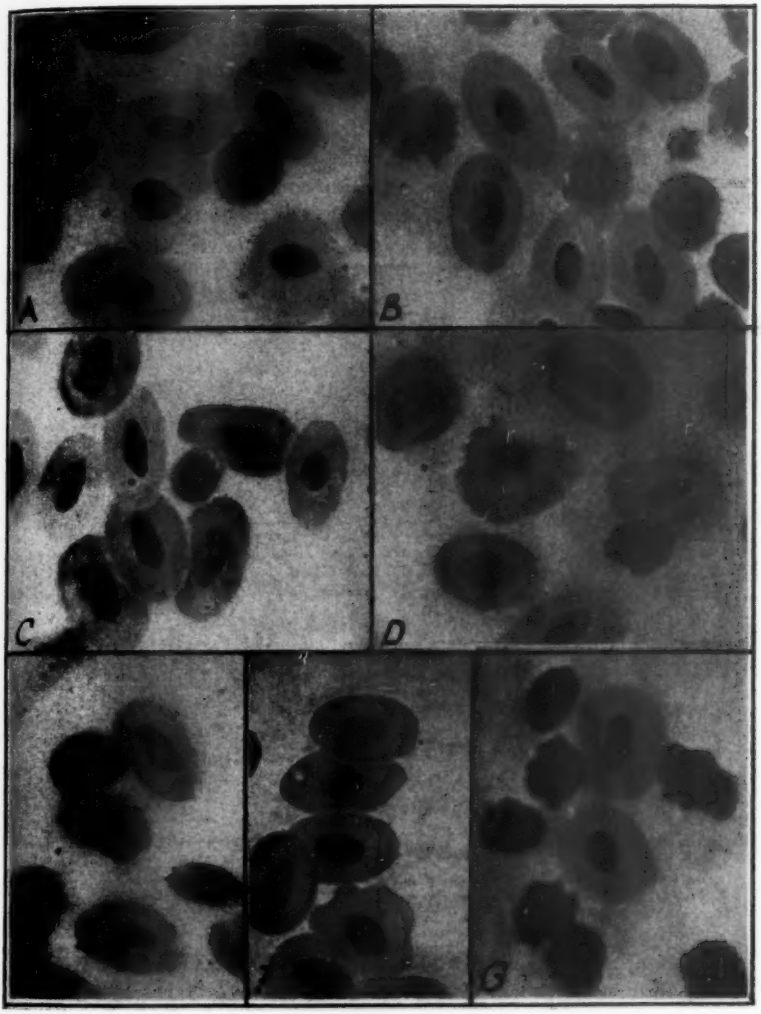

FIGURE III

A. Normal erythrocytes and their nuclei are elliptical. Their cytoplasm stains pink and the nucleus purple with a combination of Wright and Giemsa stains.
B. Injured erythrocytes swell and their nucleus stains pink.
C. Some of the nuclei become small and pyknotic following injury.
D. The cytoplasm of injured erythrocytes swells and stains faintly pink. Sometimes it is difficult to see any cytoplasm around degenerating nuclei.
E. Masses of hemoglobin sometimes are found without any nuclei. This apparently results from injury to erythrocytes.
F. Vacuoles are present in the cytoplasm of some erythrocytes.
G. Degenerating erythrocytes sometimes appear only as irregular masses of pink staining material in a smear of the peripheral blood.

11.5 cells; forty-eight hours, 4.0 cells. In thirty observations on ten normal ducks only 1.5 degenerating erythrocytes per 500 erythrocytes were found. In these observations only one specific type of injured cell was counted (Fig. III-B). However, in the same smears there were many other erythrocytes showing varying degrees of injury.

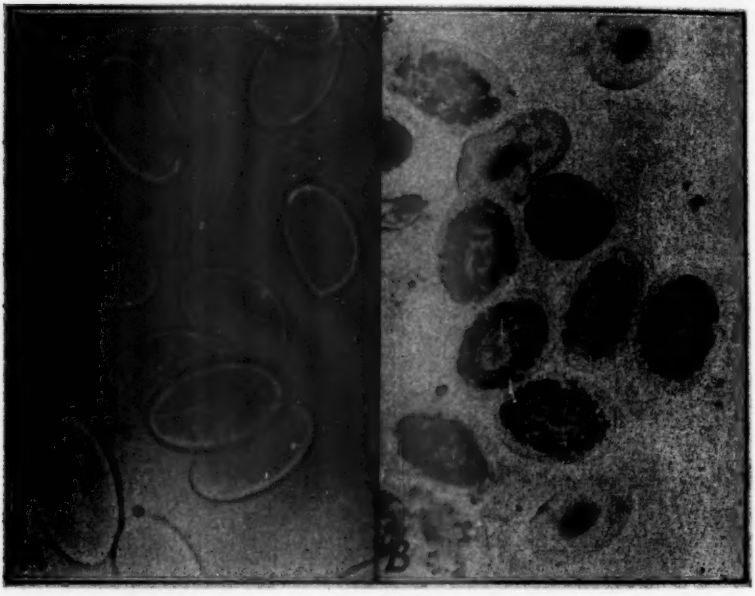

FIGURE IV

A. Normal erythrocytes in a supravital preparation stained with brilliant cresyl blue.
B. Reticulum in the cytoplasm of erythroblasts stained wth brilliant cresyl blue.

Supravital preparations of the blood from ducks given phenylhydrazine hydrochloride showed reddish-blue refractile granules within the cytoplasm. Blood from normal ducks also showed similar granules in the phenylhydrazine-treated and nontreated birds. The erythroblasts showed a basophilic, fine, reticulum-like material within the cytoplasm (Fig. IV-B). The quantity of this basophilic material was much greater in the younger erythroblasts. As the erythroblasts approached ma-

turity there was a progressive decrease in the amount of this cytoplasmic material. Some of the oval-shaped erythrocytes with elliptical nuclei showed small clumps of the basophilic material. An occasional small globular mass of normal staining hemoglobin was observed within the circulation (Fig. III-E). Apparently these masses of hemoglobin were from degenerating erythrocytes. Sometimes the margins of the erythrocytes were distorted. An occasional red cell had a small vacuole within the cytoplasm (Fig. III-F). A few of the nuclei were small and pyknotic (Fig. III-C). Fragments of nuclei were not observed either within degenerating erythrocytes or free in the plasma.

The serum was reddish-brown in color from six to forty-eight hours after phenylhydrazine hydrochloride was given. The spleen and liver were enlarged and reddish-brown in color from six to ninety-six hours following the administration of phenylhydrazine. During this time yellowish-brown granules of pigment were found in the cells of the reticulo-endothelial system. This pigment in the liver and spleen gave a negative reaction for iron in ducks killed six hours after the phenylhydrazine was given. Twenty-four hours after the phenylhydrazine was given the pigment gave a positive reaction for iron. These tissues continued to show the presence of iron for ten days. The spleen and liver from a duck killed fourteen days following the administration of phenylhydrazine were normal; no pigments were observed in the reticulo-endothelial system and the stains for iron were negative. No iron was demonstrated by the same technique in the liver and spleen of ten normal ducks killed when twenty-one days of age.

There occurred a hyperplasia of the erythroid cells in the bone marrow of each duck given phenylhydrazine[1] (Table 1). Such would be expected in the presence of the larger number of erythroblasts within the peripheral blood. The normal myelogram for the duck has been established by Crass and Rigdon (to be published).

Discussion. Little is known of the mechanism by which phenylhydrazine hydrochloride destroys nonnucleated erythrocytes, although the problem has been studied frequently as

[1] The bone marrow studies were made by Dr. Gwendolyn Crass. We acknowledge our appreciation to her for this assistance.

indicated in the review by Webster in 1949. It has been suggested that this drug affects either the protoplasm or cell membrane of the nonnucleated red cells (Webster, 1949). This opinion is based upon the presence of Heinz-Ehrlich bodies within the injured erythrocytes. No changes were observed, however, in the cytoplasm of the nucleated erythrocytes in the duck to suggest Heinz-Ehrlich bodies. Our observations on the morphologic changes in the red cell of the duck would suggest

TABLE 1

Percentage of Erythroid and Myeloid Cells in the Bone Marrow of Ducks given Phenylhydrazine Hydrochloride

Age of Duck in Days	Experimental Days	Amount PH in cc.*	Reticulocytes per 1000 RBC	RBC in M.	Bone Marrow Percent Erythroid Cells	Bone Marrow Percent Myeloid Cells
21	0 1 2	1.5 1.0 sacrificed	121	1.44	74.9	24.6
27	0 3	1.5 sacrificed	624	1.66	76.4	23.6
28	0 3	1.5 sacrificed	624	1.59	75.8	24.2
30	0 2 5	1.5 1.5 sacrificed	724	1.98	60.2	29.8
57	0 2 4	1.5 1.5 sacrificed	471	1.26	73.5	26.5
57	0 2 4	1.5 1.5 sacrificed	291	1.63	70.0	30.0
28	control				44.1	55.9
30	control				42.5	57.5
56	control				47.2	62.8
57	control				37.1	62.4

* 20 mg. per cc.

that the primary injury occurs in the nucleus. As a result of this the nucleus becomes markedly swollen and finally fragments. Accompanying this nuclear change the cytoplasm stains a lighter pink color. Usually the cytoplasm of the red cells

is invisible when the nucleus completely disintegrates. The injured erythrocytes apparently are removed very rapidly from the peripheral blood by the reticulo-endothelial system.

Few degenerating cells, like those occurring in the peripheral blood after the administration of phenylhydrazine hydrochloride, may be found in routine blood smears from normal ducks. Cullen in 1903 and Rawitz in 1899 and 1901 have observed similar degenerating cells in blood smears from dogfish, skate, and *Sargus vulgaris*. These investigators have considered the question as to whether these cellular changes occurred in the circulating blood or if they were "postmortem" changes. Cullen (1903) expressed the opinion that "it is quite natural to suppose that the oldest cells may already break down in the circulation, and we have been able to convince ourselves that such a destruction actually does go on under normal conditions in the blood of all vertebrates. In the blood of birds . . . it is always possible to demonstrate a certain number of erythrocytes which are undergoing dissolution and to make out all possible intermediary forms between the mature normaocyte and its shadow, in which the stroma is practically all gone and the nucleus remains as faintly staining hydropic mass."

Duck erythrocytes placed in water and subsequently stained with a combination of Wright and Giemsa stain show degenerating changes in their nuclei similar to those which follow the oral administration of phenlyhydrazine. Since similar change may occur in duck erythrocytes *in vitro*, as the result of aging, and following the oral administration of phenylhydrazine hydrochloride, it would be impossible to conclude which process was responsible for the injury of any specific cell; however, when there is a correlation between the time of administration of a toxic agent and the progressive increase in the number of degenerating erythrocytes in smears from the peripheral blood, one would be justified in concluding some relation between the drug, the acute anemia, and the presence of degenerating erythrocytes.

The pigment eliminated from the degenerating erythrocytes is phagocytized by the cells of the reticulo-endothelial system. It is of interest to note that the potassium ferrocyanide stain

for iron is negative during the first six hours after phenylhydrazine hydrochloride is given, although a brownish pigment may be seen in the Kupffer cells of the liver in a hemotoxylin and phloxine stain. Twenty-four hours later this pigment gives a positive reaction for iron. This would suggest that the pigments liberated from the injured red cells are acted upon in some way by the cells of the reticulo-endothelial system to convert them into a form of iron that can be demonstrated by this technique. Malarial pigment at first gives a negative stain for iron; however, with the elapse of time this pigment also gives a positive potassium ferrocyanide reaction (Rigdon, 1945).

The erythroblasts that appear in the peripheral blood following the destruction of erythrocytes by phenylhydrazine hydrochloride are similar to those previously described in malarial infected ducks (Rostorfer, 1946). It is of interest to observe the basophilic granular material within the cytoplasm of the erythroblasts. The similarity of this material in the duck erythroblast to that in the mammalian reticulocyte is obvious. Sabin, in 1927, described similar basophilic material in the red blood cells of the chick embryo.

Summary

The morphologic changes occurring in the nucleated erythrocytes of ducks given phenlyhydrazine hydrochloride are discussed. The primary injury produced by this hemolytic agent occurs in the nucleus, while the primary effect is either on the hemoglobin or cell membrane of the nonnucleated erythrocytes. Hyperplasia of the erythroid elements in the bone marrow accompany the anemia produced by phenylhydrazine hydrochloride.

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A RAPID PROCEDURE FOR DETERMINING RELATIVE ANTIBIOTIC SENSITIVITIES OF MICROORGANISMS

DAVID E. SINGER, LAURIE V. PIERCE, ELIZABETH C. YAWN, ALBERT V. HARDY, AND ROLAND B. MITCHELL*

Several methods are available for determining the *in vitro* sensitivity of organisms to various therapeutic agents. The disc method has gained wide acceptance. Bondi's (1947) original publication described technics for penicillin and streptomycin determinations. More recent studies by Fusillo (1952), Mitchell (1951), Anderson (1951), McLaurin (1951), Scott (1950), including our own, have adapted this original technic to other therapeutic agents. The values obtained by this method are relative, but this is the information needed as a guide to therapy. If true levels are desired, these may be obtained by the more time-consuming tube titration technic.

The major advantage of the "disc-plate" method is the rapidity with which results may be made available to the clinician. The report can be provided in about 18 hours in many of the cases, and under favorable circumstances even earlier than this. Dependable observations may be made prior to isolation of all organisms in pure culture. This test, in addition to being rapid, is relatively simple technically and requires a minimum amount of material.

In the test described here, the sensitivity to seven therapeutic agents, penicillin, dihydrostreptomycin, neomycin, aureomycin, chloromycetin, terramycin, and sulfadiazine—is tested on one culture plate. This procedure has been used for more than a year. The results have proved satisfactory to the clinicians and the tests were easily integrated into the routine of a busy diagnostic bacteriologic laboratory. The reliability of findings was examined by comparing observations with those obtained by quantitative tube titrations. Our findings were also compared with those obtained by using commercially prepared discs.

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Clinical opinion, laboratory experience and comparative studies all indicate that this is a procedure of high practical value.

During these studies we examined 2,818 miscellaneous bacteriologic specimens, from which 3,732 bacterial cultures were isolated and identified. The *in vitro* antibiotic sensitivities of these organisms have been determined and these findings also are presented here.

Technic for Rapid Testing of Sensitivity. The technic for rapid testing of *in vitro* antibiotic sensitivity consists of four main divisions. The first, consists of the preparation of the specimen and inoculation to agar and enrichment broth culture media as described below. The second, is the placing of antibiotic sensitivity discs on the inoculated blood and/or chocolate agar plates. The third, following incubation, is the measuring of the zones of inhibition of bacterial growth surrounding each antibiotic sensitivity disc. The relative *in vitro* susceptibility of a microorganism to the various therapeutic agents can be determined and reported on the basis of these measurements. The last step is the bacteriologic identification of the microorganism involved.

Culture plates of three different agar media and one broth tube are inoculated. One plate each of eosin-methylene blue agar (Bacto) and chocolate agar (Bacto with supplement B) are streaked for isolation. A blood agar plate (5 per cent blood) is used routinely for the antibiotic sensitivity determinations. This supports the growth of most organisms encountered in routine work; it contains no interfering dyes; and the zones of inhibition are easy to read on the red background. For the more fastidious organisms as members of the *Hemophilus* and *Neisseria* groups, chocolate agar is used for the testing of sensitivity in subcultures. Thioglycollate broth, a satisfactory medium for anaerobic as well as aerobic organisms, is also used. After applying the discs as described below, the blood agar, the eosin-methylene blue agar, and the thioglycollate broth are incubated at 37° C. while the chocolate agar is placed in 10 per cent carbon dioxide and held at 36° C. All are examined after 18 hours. If there is no evident growth, incubation is continued for another 24 hours before reporting "no growth." If there is adequate growth on all segments of the blood agar

plate, the sensitivity is evaluated and findings are reported. Subcultures are made for the identification of organisms and, if necessary, for a repetition of the sensitivity tests.

Culture plates used for sensitivity determinations should have a uniform and moderately heavy inoculation. In our hands this was best accomplished by first applying the inoculum in a vertical streak bisecting the plate and then spreading by cross-streaking with a loop. The lower one-fifth of the plate or a little less is left unstreaked, to serve primarily as a guide in applying discs. The amount of inoculum to be applied must be judged from the nature of the specimen.

Discs are prepared from Whatman No. 2 filter paper with a paper punch approximately 6.5 mm. in diameter. These are placed in a Petri dish, sealed with masking tape and wrapped to insure dryness during steam sterilization.

Stock solutions of all antibiotics are prepared as shown in table 1 by the addition of a diluent to a vial of the pure powdered or crystalline form of the drug. These solutions are kept frozen to insure stability and may be used for the preparation of working solutions throughout a three-month period without any appreciable loss in antibacterial activity with the exception of penicillin. Fresh penicillin stock solutions are made every two months.

Working solutions of the respective agents are prepared from the stock solutions as shown in table 1. These are tubed in 1-ml. amounts in sterile and corked standard 4-inch Wassermann tubes, 20 tubes per lot. These solutions are stored in the freezing compartment of the refrigerator, with one tube of each drug being removed and thawed for each day's use.

Sulfadiazine solutions (5 per cent) are prepared as shown in table 1. To minimize crystallization, these are stored at room temperature. Working solutions are weighed and prepared directly from the sodium salt, omitting the use of a stock solution.

The discs are applied by using sharp-pointed forceps, one for each therapeutic agent. Prior to use, the tips of the forceps are dipped in alcohol and flamed. The inoculated blood agar plate is placed with the unstreaked portion at the bottom, or nearest the person. In succession, the discs are saturated with

TABLE 1

Preparation of Stock and Working Solutions from Commercial Products

Agent	Stock Solutions		Amount diluent added (ml.)	Concentration of stock (/ml.)	Working Solutions		
	Initial amount	Diluent			Amount of stock (ml.)	Amount of diluent (ml.)	Concentration working solution (/ml.)
Penicillin	100,000 units	N/15 phosphate buffer, pH 6.0	20	5,000 units	0.2	19.8	50 U.
Dihydrostreptomycin	1,000,000 mcgm.	N/15 phosphate buffer, pH 8.0	5	200,000 mcgm.	0.1	39.9*	500 mcgm.
Neomycin	500,000 mcgm.	Distilled water	10	50,000 mcgm.	0.3	19.7	750 mcgm.
Aureomycin	100,000 mcgm.	Distilled water	10	10,000 mcgm.	1.0	19.0	500 mcgm.
Chloromycetin	500,000 mcgm.	Distilled water	10	50,000 mcgm.	1.28	18.72	700 mcgm.
Terramycin	500,000 mcgm.	N/10 HCL	10	50,000 mcgm.	.2	19.8	500 mcgm.
Sulfadiazine	500 mg.	Distilled water	10	5 percent	...	....	5 percent

* Discard 20 ml.

1. Crystalline Penicillin G, Sodium, Bristol Laboratories.
2. Dihydrostreptomycin Sulfate, Merck Company.
3. Neomycin Sulfate, Upjohn Company.
4. Aureomycin HCl, (Parenteral), Lederle Laboratories.
5. Chloromycetin, Crystalline, Parke-Davis & Company.
6. Terramycin Hydrochloride, Crystalline, Chas. Pfizer & Company.
7. Sodium Sulfadiazine, U.S.P., Powder, Sharpe & Dohme.

the respective solutions. The edge is touched to the solution and the disc is saturated by capillary force. Excess solution is drained by slowly drawing the disc up the tube wall. Six discs are placed in a circular pattern, 25 to 30 mm. apart, starting at the lower left of the streak. The seventh is placed in the center of the plate. By arranging the discs in this way, it is not necessary to mark them individually since their order is always the same.

After sufficient incubation, the diameter of the zones of inhibition around the disc is measured with a millimeter rule and recorded. According to these measurements, the relative degree of susceptibility is classified as very sensitive (VS), moderately sensitive (MS), or resistant (R) (see Table 2). The zone of

TABLE 2

Ranges in Diameter of Zones of Inhibition and the Corresponding Reported in Vitro Sensitivities

Agent	Diameter of zone of inhibition (mm.)	Reported in vitro sensitivity
Penicillin	20 and over	VS
	10–19	MS
	9 and under	R
Dihydrostreptomycin	16 and over	VS
	10–15	MS
	9 and under	R
Neomycin	16 and over	VS
	10–15	MS
	9 and under	R
Aureomycin	18 and over	VS
	13–17	MS
	12 and under	R
Chloromycetin	16 and over	VS
	10–15	MS
	9 and under	R
Terramycin	16 and over	VS
	10–15	MS
	9 and under	R
Sulfadiazine	18 and over	VS
	10–17	MS
	9 and under	R

VS—very sensitive.
MS—moderately sensitive.
R—resistant.

inhibition is not always defined by a fine line of demarcation, nor is it always precisely round. Thus differences of 1 or 2 mm. may represent only experimental variations. In making com-

parisons, therefore, we have recorded disagreement only when there was a difference of more than 2 mm. in measurements obtained by the use of different discs.

A sensitivity report may be rendered on initial plating in any of the following circumstances: (1) one organism is present, (2) one organism predominates in a mixed bacterial culture, or (3) multiple organisms having the same sensitivity. Subcultures are made for additional studies of the individual organisms in pure culture.

Desirable economies in materials and time of personnel may be attained if the desire of the physician or the nature of the infection is known. With this information, selection of specific chemotherapeutic agents for sensitivity determinations is possible.

Fresh and Commercially Prepared Discs Compared. Results obtained by the use of commercially prepared sensitivity discs[1] were compared with those obtained by the discs prepared as described. A variety of freshly isolated organisms from various sources were selected and 120 tests in parallel were made. There was a very close correlation in results. The comparative findings are summarized in table 3.

TABLE 3

Comparison of Sensitivity by Commercial Dry Disc and Fresh Moist Disc*

Agent Tested	Number of Cultures	Results in Agreement		Higher sensitivity shown by Dry Disc		Higher sensitivity shown by Moist Disc	
		No.	Percent	No.	Percent	No.	Percent
Aureomycin	23	22	95.6	1	4.4	0	0
Terramycin	22	20	90.9	2	9.1	0	0
Chloromycetin	31	21	67.7	8	25.8	2	6.5
Dihydro-streptomycin	21	20	95.3	0	0	1	4.7
Penicillin	23	22	95.6	1	4.4	0	0
Totals	120	105	87.5	12	10.0	3	2.5

*Aureomycin, Lederle Laboratories.
Terramycin, Chloromycetin, Dihydrostreptomycin, and Penicillin, Difco Laboratories.

Of 120 comparisons, 105 (87.5 per cent) were in agreement as to the reported sensitivity, while in 12 (10.0 per cent) a

[1] Difco, Lederle, and Parke-Davis.

higher sensitivity was shown by the commercial dry disc. In 3 (2.5 per cent) the prepared moist disc gave a higher sensitivity. Of the 15 comparisons which were in disagreement, fourteen gave only minor variation, varying between moderate and a very sensitive or resistant and moderately sensitive. In only one case did a wide variation occur, from very sensitive to resistant.

Findings by Tube Titration and Disc-Plate Method Compared. The results obtained by the described method and those using tube titrations were compared. The two methods differ widely in that the disc plate method is solely a qualitative determination while tube titration is a quantitative procedure; still there were very few discrepancies.

TABLE 4

Blood Levels and Corresponding Relative Sensitivities

Agent	Normal attainable blood levels	Relative sensitivity interpretation
	*Lowry (1951)	
Penicillin	0.16 U. or less	VS
	0.16–2.5 U.	MS
	2.5 U. or more	R
Dihydrostreptomycin	4 mcgm. or less	VS
	4–15 mcgm.	MS
	15 mcgm. or more	R
	*Duncan (1951)	
Neomycin	4 mcgm. or less	VS
	4–10 mcgm.	MS
	10 mcgm. or more	R
	*Lepper (1949)	
Aureomycin	2 mcgm. or less	VS
	2–6 mcgm.	MS
	6 mcgm. or more	R
	*Smadel (1948)	
Chloromycetin	5 mcgm. or less	VS
	5–12 mcgm.	MS
	12 mcgm. or more	R
	*Bliss (1950)	
Terramycin	2 mcgm. or less	VS
	2–5 mcgm.	MS
	5 mcgm. or more	R
Sulfadiazine	6 mg. % or less	VS
	6–15 mg. %	MS
	15 mg.% or more	R

VS—very sensitive.
MS—moderately sensitive.
R—resistant.
*References.

To compare the findings the tube titration tests must be interpreted as very sensitive, moderately sensitive, and resistant. The usual levels attained in peripheral blood following administration of drugs in doses generally used in clinical practice are given in table 4. Theoretically, if an organism is inhibited by a concentration of antibiotic which is in the lower limits of the attainable blood level, one might expect the organism to be very susceptible to treatment with that agent. If it is inhibited by intermediate concentrations of the attainable blood level, the organism would be considered only moderately susceptible, and if inhibited only by concentrations greater than levels normally attained, the organism would be regarded as resistant to that particular preparation. On this basis, comparisons between the tube titration and the disc-plate method were made.

Organisms selected for this study were freshly isolated and included a wide variety of genera and species. Tryptone broth (Bacto) was used as the medium of choice for the tube tests because of its ability to support the growth of all organisms studied and because according to Anderson (4) it is virtually free of sulfonamide inhibitors.

In preparing the dilutions, tryptone broth is added first to each of the 10 tubes in amounts as listed in table 5. The antibiotic solution, 100 mcgm. per milliliter diluted in tryptone broth, is then added to the first 3 tubes. After thoroughly mixing tube No. 3, 2 ml. are withdrawn and 1 ml. is added to each of tubes 4 and 5. This is repeated, transferring 1 ml. from tube 5 to tubes 6 and 7, and likewise from 7 to tubes 8 and 9—to the last of which 0.5 ml. is added and the remaining 0.5 ml. is discarded. Tube 10 is the control tube with no antibiotic added. To each of 10 tubes, each containing 2 ml. of the proper dilution, one drop of an 18 to 24-hour tryptone broth culture of the organism being tested, diluted 10^{-6} or 10^{-7} depending on turbidity, is added. Results are read after 24 hours of incubation. The end point is the highest dilution which does not show visible growth or turbidity. The procedure is modified for penicillin and sulfadiazine in that the highest concentrations are 20.48 units and 200 mg. per cent, respectively. Also a 13-tube battery is used for penicillin giving a low concentration of 0.01 units per milliliter.

The comparative findings by the disc-plate and tube titration method are summarized in table 6. Both tests were read after 24 hours.

TABLE 5

Tube Test Titration Procedure

Tube Number	1	2	3	4	5	6	7	8	9	10
Tryptone Broth	0	1.0	3.0	1.0	3.0	1.0	3.0	1.0	1.5	2.0
100 mcgm. per 1 ml.	2	1.0	1.0	0	0	0	0	0	0	0
Final Volume	2	2	2	2	2	2	2	2	2	2
Conc. mcgm. per 1 ml.	100	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0

There was complete agreement in the 47 tests with terramycin, and only with aureomycin did two minor variations occur. Two cultures tested with chloromycetin were very sensitive by disc and resistant by tube titration and another was moderately sensitive by disc and resistant by tube titration. In 26 instances the results were in agreement. The sensitivity interpretations corresponded in 14 cultures tested with penicillin but in 4 the tube reading was higher, although the variation was only between moderate and very sensitive or resistant and moderately sensitive. With sulfadiazine 4 organisms were found resistant by the disc method, 2 of which were moderately sensitive and 2 very sensitive by the tube titration technic. There was agreement in findings in the other 15 tests. Using dihydrostreptomycin there were two minor variations only.

TABLE 6

Comparison of Sensitivity by Tube and Disc Technics

Agent Tested	Number of Cultures	Results in Agreement		Higher sensitivity shown by			
				Tube method		Disc method	
		No.	Percent	No.	Percent	No.	Percent
Aureomycin	33	31	93.9	2	6.1	0	0
Terramycin	47	47	100.	0	0	0	0
Chloromycetin	29	26	89.7	0	0	3	10.3
Dihydro-streptomycin	39	37	94.6	2	5.4	0	0
Neomycin	33	17	51.5	16	48.5	0	0
Penicillin	18	14	77.7	4	22.3	0	0
Sulfadiazine	19	15	78.4	4	21.6	0	0
Totals	218	187	85.8	28	12.8	3	1.4

With neomycin 17 organisms were found very sensitive by both technics, but in 16 others the disc reading indicated a lower level of sensitivity. Tests with this antibiotic warrant reconsideration.

Results of Routine Sensitivity Studies. The sources of specimens examined are indicated in table 7. Of the 2,816 submitted, 9 were unsatisfactory for culture, 474 failed to show any growth of organisms, but from the remaining 2,333 a total of 3,732 strains were isolated. *In vitro* sensitivities were determined on all.

TABLE 7

Source of 2,816 Specimens Submitted for Sensitivity Studies

Source	Positive for bacteria	Negative for bacteria	Unsatisfactory for culture	Total
Urine	848	235	4	1,087
Ear (otitis externa)	646	0	0	646
Ear (others)	139	16	0	155
Pleural fluids	28	30	4	62
Abdominal fluids	16	9	0	25
Spinal fluids	26	37	0	63
Joint fluids	2	4	0	6
Prostatic fluids	5	3	0	8
Nose and throat	58	4	0	62
Eye	32	10	0	42
Skin lesions	52	5	0	57
Wounds and amputations	28	2	0	30
Bone	23	4	0	27
Vaginal secretions	13	2	0	15
Fecal and rectum	31	1	0	32
Blood	3	1	0	4
Miscellaneous exudate	334	110	1	445
*C.F.I.	49	1	0	50
Totals	2,333	474	9	2,816

*Organisms submitted for identification and sensitivity determinations.

Sensitivity findings on 1,216 bacteria isolated from 848 positive urinary cultures are given in table 8. Table 9 shows the findings on 1,212 bacteria isolated from 646 cultures of cases of otitis externa, while the data on 1,304 bacteria isolated during the examination of 839 positive miscellaneous cultures are shown in table 10. The data presented separately in these three tables are combined in table 11.

TABLE 8

Relative Efficacy of Seven Therapeutic Agents for 1,216 Organisms Isolated from 848 Urinary Cultures

Types of organisms isolated from urines	Total	Penicillin			Dihydrostreptomycin			Neomycin			Aureomycin			Chloromycetin			Terramycin			Sulfadiazine		
		VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R
Pseudomonas group	478	0	0	478	7	36	436	13	150	315	36	131	311	41	41	397	305	136	37	172	12	294
Aerobacter aerogenes	143	0	0	143	15	48	70	13	93	37	33	63	47	65	50	27	102	28	13	8	1	134
Escherichia group	168	0	2	166	30	57	81	19	124	25	32	85	51	49	79	40	136	27	5	30	3	135
Alcaligenes group	75	0	0	75	6	9	60	18	40	17	14	33	28	6	5	64	41	24	10	51	2	22
Paracolon 29911	46	0	1	45	0	2	44	2	4	40	0	1	45	3	7	36	1	2	43	1	2	43
Paracolon group	20	0	0	20	6	4	10	1	15	4	5	7	8	5	6	9	10	2	8	0	0	1
Proteus group	101	0	0	101	6	10	85	1	46	54	5	13	83	7	15	84	16	13	72	2	1	98
Alpha streptococci	18	5	4	9	0	0	18	0	1	17	14	2	2	7	4	7	13	2	3	1	0	17
Gamma streptococci	33	5	3	25	2	1	30	3	2	28	26	4	3	6	12	15	27	2	4	2	0	31
Beta streptococci	19	7	2	10	1	0	18	0	0	19	11	3	5	8	6	5	12	0	7	1	0	18
Staphylococcus aureus, hemolytic	16	5	3	8	7	6	3	7	7	2	9	3	4	7	5	4	13	1	2	2	0	14
Staph. albus, hemolytic	37	9	9	19	23	8	6	27	8	2	23	1	13	16	12	9	24	1	12	4	2	31
Staph. citreus, hemolytic	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Staph. aureus, nonhemolytic	12	2	0	10	4	3	5	6	6	0	9	1	2	7	5	0	9	0	3	2	0	10
Staph. albus, nonhemolytic	36	13	7	16	21	3	12	30	4	2	28	0	8	18	11	7	30	1	5	5	0	31
Mimeae	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1
Yeastlike	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0	12

TABLE 9

Relative Efficacy of Seven Therapeutic Agents for 1,212 Organisms Isolated from 646 Cultures of Otitis Externa

Types of organisms isolated from cultures of otitis externa	Total	Penicillin			Dihydrostreptomycin			Neomycin			Aureomycin			Chloromycetin			Terramycin			Sulfadiazine		
		VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R
Pseudomonas group *15	423	0	0	423	16	48	359	5	96	307	10	72	341	16	18	389	222	168	38	330	20	73
Aerobacter aerogenes *6	174	0	0	174	38	94	42	13	123	32	24	86	64	48	77	49	123	41	10	14	4	156
Escherichia group	39	0	0	39	9	16	14	7	27	5	2	23	13	8	18	13	27	9	3	2	0	37
Alcaligenes group	43	0	0	43	12	6	25	3	27	13	19	16	8	2	5	36	13	19	11	35	2	6
Paracolon group *1	6	0	0	6	2	2	2	1	1	3	1	2	3	1	1	4	3	2	1	0	0	6
Proteus group	53	0	0	53	2	15	36	1	18	34	0	0	53	0	6	47	2	0	51	3	4	46
Alpha streptococci *2	22	4	1	17	3	1	18	0	0	20	18	2	2	5	6	11	21	0	1	5	1	16
Beta streptococci *2	25	15	3	7	2	0	23	1	0	22	22	2	1	13	3	9	22	0	3	7	1	17
Gamma streptococci	49	2	10	37	3	3	43	2	4	43	28	14	7	5	12	32	38	5	6	3	1	45
Staphylococcus aureus, hemolytic *2	61	17	13	34	28	28	8	25	34	3	52	7	5	15	23	26	59	0	5	7	0	57
Staph. albus, hemolytic, *10	174	52	59	63	92	61	21	102	60	2	135	18	21	55	69	50	149	3	22	16	1	157
Staph. aureus, nonhemolytic	40	14	8	18	18	14	8	20	15	5	35	2	3	16	15	9	36	1	3	7	1	32
Staph. albus, nonhemolytic	81	28	18	35	47	24	10	52	24	5	70	4	7	30	27	24	74	1	6	18	2	61
Yeastlike	19	0	0	19	0	0	19	0	0	19	0	0	19	0	0	19	0	0	19	0	0	19

*Number of isolations not tested with neomycin.

TABLE 10

Relative Efficacy of Seven Therapeutic Agents for 1,304 Organisms Isolated from 839 Cultures of Miscellaneous Exudates

Types of organisms isolated from miscellaneous exudates	Total	Penicillin			Dihydrostreptomycin			Neomycin			Aureomycin			Chloromycetin			Terramycin			Sulfadiazine		
		VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R
Pseudomonas group	118	0	0	118	3	12	101	5	32	81	10	25	83	10	14	93	67	39	12	71	1	46
Aerobacter aerogenes	103	1	0	102	22	51	30	11	74	18	18	51	34	31	52	20	78	18	7	13	2	88
Escherichia group	108	0	1	107	24	51	33	2	84	22	20	54	34	31	55	22	79	17	12	11	2	95
Alcaligenes group	33	2	1	30	4	7	22	4	21	8	11	15	7	5	5	23	18	10	5	17	2	14
Paracolon 29911	11	0	0	11	0	1	10	0	3	8	0	0	11	0	0	11	0	0	11	0	0	11
Paracolon group	18	0	0	18	4	6	8	0	15	3	1	11	6	2	13	3	10	6	2	2	0	16
Proteus group	88	0	0	88	7	4	77	3	35	50	3	9	76	16	16	56	11	4	73	0	0	88
Hemophilus influenzae	7	0	0	7	2	5	0	0	1	6	4	3	0	7	0	0	7	0	0	1	0	6
Koch-Weeks bacillus	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	1
Shigella group	9	0	0	9	1	7	1	4	3	2	2	5	2	6	3	0	8	1	0	0	0	9
Salmonella typhosa	3	0	0	3	0	0	3	1	2	0	0	3	0	0	3	0	0	3	0	0	0	3
Salmonella group	26	0	0	26	1	3	22	1	13	12	5	20	1	24	2	0	25	1	0	0	0	26
Diplococcus pneumoniae	6	6	0	0	0	0	6	0	0	6	6	0	0	2	2	2	6	0	0	3	0	3
Alpha streptococci	121	61	18	42	4	6	111	4	2	115	113	10	8	64	32	25	108	3	10	36	2	83
Beta streptococci	99	67	9	33	5	4	90	3	3	93	75	4	20	52	42	5	77	10	12	14	1	84
Gamma streptococci	54	8	4	42	4	1	49	4	1	49	42	4	8	13	13	28	45	3	6	5	0	49
Staphylococcus aureus, hemolytic	155	42	35	78	39	81	35	30	111	14	126	11	18	41	78	36	135	5	15	32	2	121
Staph. albus, hemolytic	110	33	31	46	44	48	18	59	39	12	82	6	22	37	47	26	83	2	25	10	3	97
Staph. citreus, hemolytic	2	0	2	0	1	1	0	2	0	0	2	0	0	1	1	0	2	0	0	2	0	0
Staph. aureus, nonhemolytic	89	30	19	40	31	36	22	28	54	7	76	6	7	36	36	17	79	7	3	17	0	72
Staph. albus, nonhemolytic	95	32	26	37	47	24	24	58	24	13	73	6	16	41	27	27	73	4	18	13	1	81
Neisseria group	17	6	5	6	8	5	4	1	9	7	12	4	1	17	0	0	17	0	0	13	0	4
Mimeae	6	0	0	6	0	0	6	0	3	3	3	1	2	2	0	4	1	3	2	0	0	6
Yeastlike	25	2	1	22	0	2	23	1	1	23	4	0	21	3	1	21	2	1	22	0	0	25

TABLE 11

Relative Efficacy of Seven Therapeutic Agents for 3,732 Organisms Isolated from 2,333 Bacteriological Cultures

Combined isolation types of organisms	Total	Penicillin			Dihydrostreptomycin			Neomycin			Aureomycin			Chloromycetin			Terramycin			Sulfadiazine		
		VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R	VS	MS	R
Pseudomonas group *15	1019	0	0	1019	26	96	897	23	278	703	56	228	735	67	73	879	594	238	87	573	33	413
Aerobacter aerogenes *6	420	1	0	419	75	193	152	37	290	87	75	200	145	145	179	96	303	87	30	35	7	378
Escherichia group	315	0	3	312	63	124	128	28	235	52	54	163	98	88	152	75	242	53	20	43	5	267
Alcaligenes group	151	2	1	148	22	22	107	25	68	38	44	64	43	13	15	123	72	53	26	103	6	42
Paracolon 29911	57	0	1	56	0	3	54	2	1	48	0	1	56	3	7	47	1	2	53	1	2	54
Paracolon group *1	44	0	0	44	12	12	20	2	31	10	7	20	17	8	20	16	23	10	12	2	0	42
Proteus group	242	0	0	242	15	29	188	5	99	138	8	22	212	23	32	187	19	17	196	5	5	232
Mimeae	7	0	0	7	0	0	7	0	4	3	4	1	2	2	0	5	1	4	2	0	0	7
Hemophilus influenzae	7	0	0	7	2	5	0	0	1	6	4	3	0	7	0	0	7	0	0	1	0	6
Koch-Weeks bacillus	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	1
Shigella group	9	0	0	9	1	7	1	4	3	2	2	5	2	6	3	0	8	1	0	0	0	9
Salmonella typhosa	3	0	0	3	0	0	3	1	2	0	0	3	0	0	3	0	0	3	0	0	0	3
Salmonella group	26	0	0	26	1	3	22	1	13	12	5	20	1	24	2	0	25	1	0	0	0	26
Diplococcus pneumoniae	6	6	0	0	0	0	6	0	0	6	6	0	0	2	2	2	6	0	0	3	0	3
Alpha streptococci *2	161	70	23	68	7	7	147	4	3	152	145	14	12	76	42	43	142	5	14	42	3	116
Beta streptococci *2	143	89	14	50	8	4	131	4	3	134	108	9	26	73	51	19	111	10	22	22	2	119
Gamma streptococci	136	15	17	104	9	5	122	9	7	120	96	22	18	24	37	75	110	19	16	10	1	125
Staphylococcus aureus, hemolytic *2	235	74	51	120	74	115	46	62	152	19	187	21	27	63	106	66	207	6	22	41	2	192
Staph. albus, hemolytic *2	321	94	99	128	159	117	45	98	107	16	240	25	56	108	128	85	256	6	59	30	6	285
Staph. citreus, hemolytic	3	0	3	0	2	1	0	3	0	0	3	0	0	2	1	0	3	0	0	3	0	0
Staph. aureus, nonhemolytic	141	46	27	68	53	53	35	54	75	12	120	9	12	59	56	26	124	8	9	26	1	114
Staph. albus, nonhemolytic	212	73	51	88	115	51	46	140	52	20	171	10	31	89	65	58	177	6	29	36	3	173
Yeastlike	56	2	1	53	0	2	54	1	1	54	4	0	52	3	1	52	2	1	53	0	0	56
Neisseria group	17	6	5	6	8	5	4	1	9	7	12	4	1	17	0	0	17	0	0	13	0	4

*Number of isolations not tested with neomycin.

Except for the *Pseudomonas* group, similar types of bacteria isolated from different sources were very similar as to sensitivity. Isolated from urine, 61.7 per cent of the *Pseudomonas* group were completely resistant to sulfadiazine; from miscellaneous exudates, only 38.9 per cent were resistant; from otitis externa, 16.5 per cent were resistant. Terramycin appears to be the drug of choice for members of the *Pseudomonas* group, and in contrast with sulfadiazine there was no variation in sensitivity depending on source.

Only a few strains of *Proteus* were sensitive to the preparations tested, although dihydrostreptomycin, neomycin, and chloromycetin appeared promising in about one-third of the isolations. Considering the other gram-negative organisms, terramycin gave the best *in vitro* results followed by neomycin, chloromycetin, aureomycin, dihydrostreptomycin, sulfadiazine, and penicillin in order of effectiveness. For both streptococci and staphylococci, terramycin followed closely by aureomycin had the most favorable action. Although a relatively few diplococci were isolated, penicillin, terramycin, and aureomycin appeared to have equal promise. Chloromycetin and terramycin gave the best results with members of the *Neisseria* group which were tested.

Summary and Conclusions

A rapid "disc-plate" method for determining relative sensitivity of microorganisms to seven therapeutic agents is described. Results adequate for providing clinical guidance in therapy can be reported frequently in less than 24 hours and in most cases in less than 48 hours. Delays beyond 24 or 48 hours are due to the necessity of replating.

The disc-plate method gives results which compared closely with those obtained by the use of discs available commercially. A comparison of sensitivities determined by tube titration and the disc-plate method gave observations which were in close agreement, but with significant variations when using sulfadiazine and neomycin.

Sensitivity tests were performed on 3,732 organisms isolated from urine, otitis externa, and miscellaneous exudates, and the findings are reported. Sensitivities of the same variety of microorganisms were found to be essentially the same, regardless of

source, with the exception of members of the *Pseudomonas* group.

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THE USE OF POLYVINYLPYRROLIDONE AS A PLASMA VOLUME EXPANDER IN PREVENTING OR COMBATING SHOCK

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It is generally agreed that blood given intravenously to restore the circulating blood volume is the best agent that we have in treating shock, especially if the shock is produced or complicated by hemorrhage. Although blood is now readily available, excessive demands resulting from war or other catastrophies may make the present supply wholly inadequate. Since stockpiling of blood is impossible because of its perishable nature, there has been an extensive search for an adequate substitute.

Blood derivatives such as plasma, lyopilized plasma, and serum albumin can be stored, but since they must be obtained from human donors the supply necessarily will be limited. The incident of homologous serum jaundice transmitted by these blood derivatives is greatly reduced but still remains a problem.

Before blood became so readily available the collection and storage of ascitic fluid from patients with portal hypertension was advocated. An ascitic fluid bank was established at Charity Hospital in New Orleans by Maes and Davis (1941). Although they reported good results with no bad reactions this bank has been discontinued since blood has become plentiful.

We have attempted to utilize ascitic fluid obtained from cirrhosis cases in this institution in a similar manner but the technical difficulties in collecting and processing seem to outweigh the value, especially with a source of supply which is very limited when compared with that of blood and blood products.

Plasma substitutes have been investigated by many workers in recent years. The effectiveness of these plasma extenders or plasma volume expanders is based on the well established principle that certain exogenous macromolecular colloids are capable of increasing and maintaining blood volume by means of their

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osmotic pressure, hydrophilic properties and slow elimination from the vascular system. The colloid must be nontoxic, nonantigenic and stable, and inexpensive to make from readily available material. Accumulation and retention of the colloid sufficient to interfere with vital functions must not occur.

The plasma substitutes thought to have the greatest usefulness at the present time consist of the modified proteins (oxypolygelatin and modified fluid gelatin), polymerized carbohydrates (dextran) and the synthetics or plastics (polyvinylpyrrolidone). While none of these are considered to represent an ideal preparation the general feeling is that they should be stockpiled for an extreme emergency, such as an atomic war, while further investigation for a better substitute goes on. Even if a perfect plasma expander is found it is doubtful that it could replace the utilizable protein, immune bodies, cellular elements, etc., which are present in whole blood.

Our own experience with plasma expanders has been limited to the use of polyvinylpyrrolidone (P.V.P.). Under the name "Kollidon" this blood substitute was developed by Hecht and Weese during World War II in Germany where the supply of human blood was very limited. This water soluble polymer is a whitish, amorphous powder prepared by controlled polymerization of vinylpyrrolidone, a product of acetylene chemistry, under special high pressure, high temperature technique. This powder, dissolved in water to make a 2.5 per cent solution together with physiological inorganic salts and sterilized by heat, was called Periston. At the present time P.V.P. is supplied in a 3.5 per cent solution, since Lundy (1950) and others found this concentration more effective in maintaining blood pressure. P.V.P. develops a colloidal osmotic pressure of 300–350 mm. of water. It has a pH of 5.7–7 and a viscosity 2–3 times greater than water. The average molecular weight is approximately 40,000 (K–30). P.V.P. is nonantigenic and nonallergenic in man, although reactions have occurred in dogs. It requires no blood grouping nor crossmatching and may be sterilized, stored and administered without special precautions. P.V.P. does not interfere with blood formation, blood coagulation, or the oxygen-carrying power of the blood. The sedimentation rate, however,

usually is increased, and often the urine will show a positive test for albumen.

P.V.P. has been advocated not only as a blood substitute but also as a retardant vehicle for drugs and as a transporting and detoxifying vehicle.

The main concern in the use of polyvinylpyrrolidone has been its ultimate disposition. This function is far from settled. Twenty to 50 per cent of P.V.P., representing the polymer fraction of lowest molecular weight, is filtered out by the kidney three days after administration, and has been detected in the urine up to 10 days after injection though only in very small amounts. A part of the nonexcreted portion appears to be taken up slowly by the reticuloendothelial system in the liver, spleen, kidneys, endothelium of blood vessels, and lymph-nodes. The fate of the remainder is unknown. The parenchyma of organs does not appear to be damaged, however, since vital functions are not affected.

During World War II the Germans supposedly used P.V.P. in 500,000 cases without bad results, though this has been difficult to substantiate in detail. Ravdin (1952) reported that there was no significant interference with hepatic or renal function even months after the intravenous injection of P.V.P. in amounts up to 3,000 cc. Clark (1951) found no ill effects after treating 33 patients with amounts of P.V.P. ranging from 400 cc. to 2,000 cc. given as often as three times in 30 days. Lundy (1951) has been able to support blood pressure satisfactorily during anesthesia in 200 cases, the P.V.P. being used in quantities as small as 300 cc. There were no untoward effects.

Arden (1951), Pagani (1950), Weese (1951), and others, have also reported good results without harmful after-effects. Weese stated that only in infants and test animals is significant storage observed following administration of doses in excess of therapeutic requirements. Hartman's (1951) experiments tended to show that P.V.P. was stored more rapidly and in greater quantity than gelatin, dextran or pectin, but only after it had been given in repeated or large injections.

It should be mentioned that polyvinylpyrrolidone can be manufactured with varying average molecular weight. A correlation exists between viscosity and average molecular weight

of a mixture of polymers ("K" value). Preparations with a K value of 25 can be excreted almost totally in two to four days, in contrast to the original preparations (K30) which are not excreted in toto. A preparation with lower K value which is effective but leads to less retention, may be found in the future to be more desirable.

At the John Sealy Hospital P.V.P. (Macrose) has been used to treat shock or impending shock in 52 patients. These patients ranged in age from 14 to 75 years. The P.V.P. treatment was given chiefly in the emergency room. In cases where shock was severe or where a large amount of blood had been lost, whole blood was given later. The amount of P.V.P. given ranged from 500 to 1,500 cc. per patient. There were no reactions or ill effects attributable to P.V.P. Four patients died, but they were considered to be in a terminal state at the time of administration of the P.V.P. The causes of death were heart failure (two patients), acute necrosis of the liver, and peritonitis.

Twenty-seven cases were in mild or impending shock. Twenty-five of these were benefited as judged by rising blood pressures and clinical improvement. Many of these would probably have responded without therapy. Two of the twenty-five also received blood, but improvement had been evident before the blood was started. The other two cases improved only after blood was given.

Nineteen of twenty-five cases in moderate to severe shock were brought out of shock, thereafter normal blood pressures were maintained. Six of the nineteen received blood following the P.V.P. administration. One case did not respond to P.V.P. but did to whole blood. Another showed no immediate response to P.V.P. or to whole blood, but eventually did emerge from shock.

The four patients who died were in a terminal state and did not respond to any therapy. Probably these four should not be included in this evaluation. Extensive laboratory tests were not carried out in these cases but the data that were obtained showed no abnormalities that could be attributed to P.V.P. administration.

One patient with extensive anasarca and hypoproteinemia was given 1,000 cc. of P.V.P. in the hope of increasing the

vascular osmotic pressure and reducing the edema. There was no apparent benefit.

The P.V.P. used in our study was supplied through the courtesy of the Schenley Laboratories of New York. The preparation contains 3.5 per cent (by weight) polyvinylpyrrolidone, 0.65 per cent sodium chloride, 0.042 per cent potassium chloride, 0.025 per cent calcium chloride, 0.0005 per cent magnesium chloride, 6 H_2O, and 0.024 per cent sodium bicarbonate.

Summary

Polyvinylpyrrolidone was administered to 52 cases of impending or real shock without discernible bad effects and with apparent benefit in 44 cases. This experience is reported to add to the data on plasma volume expanders. The threat of extensive casualties from atomic warfare makes the finding of a satisfactory expander essential in order to reinforce and to extend the supply of whole blood, which of itself would be in entirely inadequate supply in such an emergency. Such expanders also may be used as an economy measure, to sustain blood pressure during operations or to treat mild shock in cases in which blood loss is not an important factor.

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A SURVEY OF TYPHUS IN RATS AND RAT ECTOPARASITES IN GALVESTON, TEXAS*

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In January of 1945 a survey of a small sample of rats in Galveston indicated a rather high incidence of murine typhus. Consequently a trapping campaign was initiated by Mr. A. L. Harris and the senior author. In September of 1945 the United States Public Health Service provided men and materials for a rat trapping program in Galveston in cooperation with the state and city health departments. Hereafter rats were trapped throughout the city by personnel of the U.S.P.H.S. and brought to our laboratory for processing. We were interested in finding what per cent of rats harbored murine typhus antibodies, whether we could recover the virus from the ectoparasites by inoculating them into laboratory animals, if we could transmit typhus from rat to rat with laboratory cultures of mites, and, finally, in a simple survey of the number and kinds of rat ectoparasites.

Acknowledgments. A great many individuals have entered into this survey in one way or another and it would be impossible to mention all who have helped. We thank especially Drs. Ludwik Anigstein and Madero N. Bader for invaluable technical assistance and criticism, H. B. Morlan, Louis J. Ogden and members of the state and city health departments for channeling the material through our laboratory, and Arthur Lee Harris and Carrie Lee Brown (now Mrs. Sullivan) for their very active interest and cheerful assistance in this project.

Blood Serologies. An effort was made to get sufficient blood from every rat brought into the laboratory to make a complement fixation test for typhus. Some rats were dead before they were brought in, some failed to yield blood even after repeated efforts of the entire lab force, and occasional vials were broken, so that the number of tests are less than the total number of rats caught.

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In 1945, 141 blood samples were collected: 63 per cent were positive for typhus antibodies, 30.5 per cent were negative and 6.5 per cent were anticomplementary. In 1946, of a total of 131 samples, 58.8 per cent were positive, 33.6 per cent were negative and 7.6 per cent were anticomplementary. In 1947 there were 110 samples (first seven months only) with 32.7 per cent positive, 54.6 per cent negative and 12.7 per cent anticomplementary.

The rats were collected from all regions within the corporate limits of Galveston, business and residential areas alike. The same regions were represented each year. We believe the sharp drop in positive rats in the first seven months of 1947 was due to an intensive program of DDT dusting initiated about the middle of 1946. As will be shown later, there was a sharp decrease of fleas per rat after the dusting program got into full swing and the decrease in positive rats was the probable natural result.

Natural Infections of Ectoparasites. One of the objectives of the survey was to determine so far as possible how many of the various ectoparasites of rats were naturally infected with murine typhus rickettsia. Our method of surveying this was as follows. The rat was anesthetized with chloroform and the ectoparasites brushed off the rat into a white enameled pan. If the chloroforming was properly timed, the parasites dropped off readily but rapidly recovered. They were then collected into sterile physiological saline and sorted to species under a dissecting microscope. Each batch to be inoculated was washed twice in sterile saline and then placed in a glass tissue grinder and macerated in 10 cc. of sterile skimmed milk. This was then strained through sterile gauze and inoculated intraperitoneally into young male guinea pigs. Five cc. was the standard inoculum, regardless of the number of ectoparasites used. Results were judged by the standard fever and scrotal reactions, followed either by immunity tests or complement fixation test of the blood. In 1945 the recovered guinea pigs were tested for immunity by subinoculation with a strain of murine typhus maintained in guinea pigs by the Fourth Army Hospital, Fort Sam Houston. In 1946 the blood of all test animals was routinely tested for antibodies by the complement fixation method,

as was also the case in 1947. The results of these tests are discussed below.

Fleas. By far the commonest flea found on rats during the two and a half years of the survey was the oriental rat flea, *Xenopsylla cheopis.* Seventy-four inoculations were made of which nineteen were positive. The common rat flea, *Leptopsylla segnis* was inoculated sixteen times and two positive results obtained. The stick tight flea, *Echidnophaga gallinacea* was inoculated fifteen times with one positive result. The dog and cat flea, *Ctenocephalides canis* and/or *felis,* was never abundant and the two inoculations made with it were negative.

Mites. Nineteen inoculations were made with the tropical rat mite, *Bdelonyssus bacoti,* and all were negative. This mite sometimes enters offices and homes of Galveston and is a great nuisance when it does. It attacks readily and its bite is variously described as painful, burning or itching, but apparently there is no prolonged effect nor any secondary reactions. In two homes that the senior author visited at the invitation of the owners, he found the mites crawling on the walls, doorsills and furniture. In one home they were most abundant on one wall against which a child's bed had been pushed. The mites crawled freely onto the bed and the child had been severely bitten. In both these homes a thorough application of DDT and pyrethrum from an insecticide bomb gave effective control. An influx of these mites probably occurs when rats on which they normally feed have been exterminated. Because this mite readily bites man we were especially interested in trying to recover rickettsia from it. We did get one questionable positive result but it was very mild and we were unable to pass it. However, Liu (1947) and others have reported the isolation of murine typhus from this mite and it should probably be considered a potential vector. Another common mite, Nuttall's rat mite *(Laelaps nuttalli),* was inoculated twenty-nine times with all negative results.

Lice. Seventeen inoculations during the two and a half years were made with the rat louse, *Polyplax spinulosa.* All results were negative.

Laboratory Transmission of Typhus. We established laboratory colonies of both *Bdellonyssus bacoti* and *Laelaps nuttalli*

and attempted to pass murine typhus from rat to rat. We tried this by inoculating a rat intraperitoneally with typhus and then introducing it into the colony and leaving it for two weeks. At the end of this time from twenty-five to seventy-five mites were collected from the colony, put in a clean cage and a clean rat introduced. Our results were all negative.

Neither were we able to pass the rickettsia by inoculating the mites into clean rats after they had been allowed to feed for two weeks on an experimentally inoculated rat. We do not claim these results to be conclusive but we are of the opinion that neither of these two mites are involved in murine typhus transmission.

Ectoparasites. Rats were brought into our laboratory in cotton bags so that no ectoparasites were lost after the rats were removed from the trap. All rats dead in the trap were discarded. As already stated, we anesthetized the rat lightly, bled it and then brushed it vigorously with a stiff tooth brush. Those parasites which were alive and vigorous were picked up with a camels hair brush into sterile saline and later inoculated. All others were washed into 70 per cent alcohol and counted at leisure. We made no special attempt to collect mange or itch mites but we did look into the ears of all rats for larval Trombiculidae. Occasionally phytophagus or predaceous mites were recovered but we considered them as accidental records.

Fleas. The flea most commonly found was the oriental rat flea, *Xenopsylla cheopis.* This is generally considered to be a summer species but we found it uniformly abundant throughout the year. It was about equally abundant in 1945 and 1946. In 1945 it averaged 7.4 per rat with an average of 67 per cent of rats harboring it. In 1946 the mean was 6.4 with 58 per cent of rats infested. By 1947 the DDT dusting program had begun to take effect and the flea index tumbled. The mean dropped to 1.13 per rat and only 15 per cent of rats were infested.

The common rat flea *Leptopsylla segnis*, was a poor second in abundance. It averaged about one per rat in 1945 and 1946, and, like all other fleas, showed an abrupt decrease in 1947. The records we have indicate that it is very definitely a winter species. A list of the parasites taken from 190 rats captured and processed in 1933–34 offers some interesting comparisons. At

that time *Xenopsylla cheopis* averaged only 1.18 per rat and was less abundant than *Leptosylla segnis*, which averaged 1.45 per rat.

The stick-tight flea *Echidnophaga gallinacea*, was never very abundant but did occasionally build up a very large population on individual rats. *Ctenocephalides canis* (and/or *felis*) was found fairly consistently but was never abundant. Only two specimens of *Ceratophyllus fasciatus* and one specimen of *Pulex irritans* were taken during the survey.

Lice. The survey had gone well into its second year before we realized that we were dealing with two species of lice instead of one, *Hoplopleura oenomydis* and *Polyplax spinulosa.* However, by far the most abundant form was *Polyplax spinulosa.* The infestation per cent was high, about 75 per cent, and remained so through the first seven months of 1947. The DDT dusting which was so effective on fleas had no apparent effect on lice. Our records indicate that lice were slightly more abundant in the winter months.

Mites. Laelaps nuttalli was the most common species of mite that was taken during the survey. It was equally abundant throughout the year and showed only a slight decrease in 1947. About 80 per cent of rats harbored this mite for an average of about 30 per rat.

Bdellonyssus bacoti was next in abundance. It is supposed to be a winter species and it did show a noticeable drop during the late summer and fall. There was no apparent decrease in 1947. About 40 per cent of the rats carried this mite for an average of only 2 per rat. The hair-clinging *Radfordia ensifera* was quite regularly recovered but was never abundant.

We made no special effort to take mange or itch mites but we did occasionally recover both *Psoroptes* sp. and *Sarcoptes* sp. Laval Trombiculidae were found fairly often in the ears of rats. They were identified by Dr. G. W. Wharton of Duke University as *Eutrombicula masoni.*

Laelaps echidninus was a common mite in 1933–34 according to records of the survey made at that time, but it was almost completely absent during the 1945–47 survey. However, it was found on rats captured on the mainland nearby and was quite

abundant in other Texas areas during the time of our survey. (See Randolph and Eads, 1946.)

Maximum Numbers of the More Common Parasites. During the course of the survey we sometimes took unusually large numbers of a parasite from a single rat. We think this is of sufficient general interest to record. *Xenopsylla cheopis*, 116 found on one rat in February of 1945 and 120 on another in July, 1946. The rat harboring 120 was completely devoid of other ectoparasites. The next two highest numbers of this flea were 87 and 77. *Leptopsylla segnis* was seldom abundant. The two highest numbers were 66 and 67 per rat.

Echidnophaga gallinacea was generally not abundant although we have several records of 20 to 40 per rat. The largest number was 650 on one rat. Many of these were firmly attached about the head and whisker region but the majority were unattached and crawling all over the body. This rat was trapped in a badly infested chicken yard and it is quite possible that the infestation was this heavy because the fleas climbed on the rat while it was confined. (See also Randolph and Eads, 1946.)

Ctenocephalides felis (and/or *canis*) was seldom represented by more than 3 or 4 specimens per rat but one host had 57.

Bdellonyssus bacoti is often unbelievably abundant in nesting areas but there are rarely ever a very large number on any one rat. The highest number that we recorded was 292. Most records ran below 20 per rat.

Laelaps nuttalli is often very numerous on rats. We have two records of over 900 per rat (946 and 903). In addition there are several records of over 500 per rat and many of over 200. A few crude experiments on longevity indicated that this mite does not live long off the host (they died within 48 hours) and this probably accounts for such heavy populations on the rats. *Bdellonyssus bacoti*, on the other hand, lives for weeks off the host and is probably never on it except for the brief intervals of feeding.

Polyplax spinulosa, several records of over 500 per rat, four of over 1,000. The highest number recorded was 4,973 from an old adult rat which did not seem to be in unusually poor condition.

The greatest number of species of ectoparasites recorded from one rat was eight. This included three fleas, four mites, one louse. One rat harboring seven different species was relatively heavily parasitized by each of them.

Discussion and Summary

A survey of murine typhus among rats and ectoparasites of rats was conducted in Galveston, Texas, from February, 1945, through July, 1947. Only Brown rats, *Rattus norvegicus*, were captured. The complement fixation test on rat bloods gave the following results: In 1945, 63 per cent of 141 rats were positive for typhus; in 1946, 58.8 per cent of 131 rats were positive; and in 1947, 32.7 per cent of 110 rats were positive. The 1947 reduction was very probably due to a city-wide DDT dusting program which materially reduced the number of rat fleas.

Small lots of ectoparasites inoculated intraperitoneally into laboratory animals to test for typhus gave the following results: Oriental rat flea, 74 inoculations, 19 positive; common rat flea, 16 inoculations, 2 positive; stick-tight chicken flea, 15 inoculations, one positive; cat flea, two inoculations, both negative; Nuttall's rat mite, 29 inoculations, all negative; tropical rat mite, 19 inoculations, all negative; louse, 17 inoculations, all negative.

The oriental rat flea *(Xenopsylla cheopis)* is probably the chief vector of typhus among rats.

Dusting rat runs and rat harborages with DDT materially reduced the number of fleas without materially affecting mites and lice. The reduction in fleas was accompanied by a reduction of positive rats and also a marked reduction in cases of human typhus.

Seasonal variation in abundance was not apparent in the oriental rat flea but the common rat flea *(Leptopsylla segnis)* showed a definite decrease in late summer and fall. The tropical rat mite *(Bdellonyssus bacoti)* also decreases in number during summer and fall but Nuttall's rat mite *(Laelaps nuttalli)* is equally abundant throughout the year. Lice were the

most numerous and most evenly distributed rat ectoparasites. There was no apparent seasonal variation in their abundance.

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CURRENT VIEWS ON PREMEDICAL EDUCATION*

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Premedical advisors in liberal arts colleges of the United States have always been confronted with the problem of just what courses to advise the premedical student to take. Opinions have changed considerably during the past twenty years and have been influenced to a great extent by so-called modern trends in education, by competition for entrance into medical school, and by the compound result of these two: a change in admission requirements of medical colleges. Diversity in policies regarding the academic advising of premedical students calls for a re-evaluation of these policies in the light of recent developments in medical and premedical educational theories.

A study was undertaken to determine just what subjects or courses a premedical advisor should recommend to his students. This was done by first examining and comparing the academic admission requirements of the seventy-nine medical colleges in the United States. Obviously, these requirements must be met before a student can be considered as an applicant for medical school. In addition, these requirements along with courses listed as recommended by medical schools reflect to some degree the current views of medical educators on what is basic to the study of medicine. However, a survey of the literature on premedical education is a much better index of the current thinking on this matter by medical and premedical educators. The results of such a survey are presented in this paper.

A third approach to determining the contents of a premedical curriculum was made by analyzing the transcripts of five hundred physicians now practicing medicine in Texas. This was done on the assumption that physicians now practicing medicine had apparently been successful in their medical studies.

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It is reasonable to assume that their premedical education prepared them adequately for the study of medicine. This, of course, does not imply that their premedical education was the best possible preparation for the study of medicine, but it does reflect current ideas on premedical education. This analysis will be the subject of a later paper.

In surveying the literature, consideration was first given to the definition and the objectives of premedical education. This was done on the premise that once the objectives are determined for premedical education, specific courses can be selected with greater facility in order to achieve these objectives. General education has received wide approval as meeting the needs of premedical students. In the discussion of general education as premedical education, the conflict over how much emphasis should be placed on science in the premedical curriculum becomes apparent. This deserves some attention. The last two sections of this paper are concerned directly with the advice of medical educators regarding specific courses to be included in the premedical student's program of studies.

The Definition of "Premedical" Education. Before beginning a discussion of the aims and objectives of premedical education, it is wise to determine just what "premedical" education is. The terms "premedical" and "preprofessional" when used to describe the education of the premedical student may give the impression that the education of the premedical student is different from the education of any other student. Dr. Fred C. Zapffe (1939), Secretary of the Association of American Medical Colleges, expresses the general attitude of medical educators regarding "premedical" education when he says that "Medical educators have gone on record as being opposed to 'pre-medical' education in any form. They even do not like the term. They did not coin it; the college did." Sweet (1944) agrees with the opinion that there is no such thing as "premedical" studies. In arguing that the aim of the college years of the premedical student is to obtain a general education, Dr. Willard C. Rappleye (1940), Dean of the Faculty of Medicine of Columbia University, says, "The college preparation should not be regarded as pre-professional or pre-anything, but a culmination of general education." In his 1950 report to the president of

Columbia University, Rappleye recommended the abolition of "premedical" education.

The term "premedical" has a tendency to give the impression of vocationalism. Zapffe (1940a) condemns this in saying that any course of studies that smacks of vocationalism fails to give a proper preparation for medicine. Another condemnation of "premedical" education comes from Gilmore (1936), who says, "The organization of so-called pre-medicine, pre-law, or pre-anything has no place in the philosophy of a liberal education." Following this trend of thought, the University of Pittsburgh enrolls its students in regular liberal arts courses rather than in a specific premedical curriculum (Crawford, 1936). Calvin and Hendrix (1946) consider the term "premedical" misleading when applied to the science courses of the college curriculum. They feel that courses designed primarily for premedical students are likely to be taught on a lower level. Calvin and Hendrix would like premedical students to be getting as much chemistry and physics as students majoring in these fields.

It seems well agreed then that a premedical education is not a particular type of education but is the term used to apply to the college education of students who will later enter medical schools. In discussing premedical education then, it must be borne in mind that it is not a particular curriculum which is under discussion but rather the college education of the premedical student.

The Objectives of Premedical Education. The discussion of the previous section implies that the education of premedical students should not be vocational training. What, then, is the purpose of premedical education? What do medical educators expect the student to get during his college years?

Actually, the premedical program of studies is vocational in so far as it must prepare the student for entrance into medical school. It must include training in scientific subjects adequate to meet the demands of medical schools (Setterfield, 1946). However, there is much concern over the problem of whether students are being trained or educated in the undergraduate school (Keighton, 1946). Zapffe (1940a) believes that students at present are trained, but not educated. Keighton (1946) quotes

Flexner as saying that "It is true that most physicians and most lawyers are mere craftsmen; it is even true that their training largely occupies itself with teaching them how to do things." It becomes necessary, then, to distinguish between training and education and to define the objectives of the educational program of premedical students. The medical school is the technical training school of the medical profession. It has the responsibility of developing skill in the application of the sciences to the practice of medicine (Keighton, 1946). The purpose of the premedical curriculum differs greatly from this objective.

The aims of the premedical program of studies have been stated in various ways by medical educators. Setterfield (1946) made a study of the opinions of both medical and premedical educators regarding the objectives of premedical education. He summarizes these opinions under three headings: (1) adequate science education, (2) an understanding of the world and human nature, and (3) emotional adjustment and maturity of the student. For Keighton (1946), the primary function of the liberal arts college with respect to premedical education is "to provide a fertile soil in which culture and idealism may root and develop." Reinke (1936) identifies the objectives of a premedical education with the objectives of the liberal arts curriculum. These are classified by him as training of the mind, development of the will, and appreciation of the beautiful in nature and art. Five aims of premedical education are listed by Calvin and Hendrix (1946). They want each student to derive the following benefits from his college education: (1) a knowledge of the fundamentals of science; (2) an appreciation of the correlation between the various branches of science; (3) a skill in using science as a tool; (4) a spirit of research; and (5) an appreciation of his responsibility to society. The chief values of the premedical program of studies, according to Stuit and Schlicher (1948), are the acquisition of good work habits, an appreciation of scholarship, the development of the scientific attitude, and an understanding of the world and its inhabitants. Rappleye (1950) expresses the objective of preprofessional education broadly by saying that it should be to prepare the student not for medicine but for life.

Three medical educators place emphasis on the development of the ability to think clearly and logically as the aim of premedical education. Sweet (1944) states this simply as "somehow learning to think." Levir (1939) calls for more emphasis in the liberal arts college on the development of habits of thought and in the ability to translate knowledge into wisdom. Zapffe (1939) expresses the objectives as "learning how to think, how to reason, and how to marshal what one knows to the end that may arise."

The purpose of premedical education is approached from the viewpoint of qualities or attitudes of a good physician by three other medical educators. Gilmore (1936) cites two main qualities expected in a good physician: (1) mastery of the science of medicine and of the application of that science; and (2) culture and refinement. He expects the liberal arts college to provide for the achievement of these aims. Four attributes of a good physician are given by Weaver (1948). He says that a doctor must be a good citizen and that this requires a cultural education; secondly, the physician must possess the tools and knowledge he needs to recognize and prevent or treat disease; thirdly, he must possess the spirit of research; and fourthly, that the physician must have a strong social consciousness. In discussing the part of the liberal arts college in the education of physicians, Fisher (1950) states that the major job of college should be to develop virtues of honesty, accuracy, intelligence, memory, application, intellectual curiosity, charity, faith, humility, hope, and patience.

In summary, it seems that the general opinion of medical educators is that the objectives of the education of premedical students are no different from the objectives of the education of any other student. Courses taken in the premedical curriculum should aim at the development of all the faculties of the student and at the same time include such subjects as are basic to the study of medicine.

General Education as Premedical Education. The proposal of general education as suitable to meet the objectives of premedical education is widespread among medical educators, but few are careful to define what they mean by general education. The terms "general," "liberal," "cultural," and "broad"

are used frequently in describing the type of education desired for premedical students without limiting the meanings of these terms.

Perhaps the most precise definition of general education given by any of the medical educators is that of Rappleye (1950). In his consideration of general education he says:

> What is meant (by general education) is orientation of the student in the several major fields of knowledge with the principles of which every truly educated person should be familiar and, in addition, reasonable mastery of one of these fields of learning. The particular field of concentration is immaterial.

Ivy (1948) attempts to get at the meaning of "liberal." He contends that "Education to be liberal or democratic must have a conscious intellectual design, because the terms 'liberal' and 'democratic' imply purpose." By way of explanation he characterizes a liberally educated person as knowing what he is doing and saying, performing his work skillfully, loving the virtues, and organizing his appetites into a disciplined army of purposes and principles.

Other writers, while not explicitly defining what they mean by general education, do conform to Rappleye's idea that it includes work in several major areas of study. However, all do not agree that there is to be a major field such as Rapplye (1950) implies. To be noted here is Levir's (1939) warning against early specialization in the undergraduate college. Conforming to the opinion that general or liberal education includes study in various areas rather than specialization in one to the exclusion of others are Anderson (1950), Weaver (1948), Fisher (1950), and the University of Colorado School of Medicine (1949).

McGrath (1945) and Calvin and Hendrix (1946) agree that the education desired for premedical students is essentially the same as the education desired for any other professional field and that it is attainable by a program of general education. This contention that general education provides principles basic for all educated men is supported by Zapffe (1940b) who says, "The first essential for general education is to determine what is fundamental and what can be taught in the time available."

The Council on Medical Education and Hospitals of the American Medical Association (1950) issued the statement that "The Council and the medical schools strongly urge that he [the premedical student] arrange his program so that he will obtain a broad general education." The State University of Iowa College of Medicine (1950) makes a similar plea to its applicants. The University of Texas Medical Branch (1950) advises students to be well-trained in the basic sciences and also to have as broad a cultural background as possible to maintain best the traditional standards of the medical profession. In Great Britain, Crowther (1935) sets a high value on a good general education. The consensus, then, among medical educators is that general education is the preferred form of education for the premedical student and that this is not essentially different from the education desired for other students.

Science Versus Nonscience Courses in the Premedical Curriculum. Granting that the premedical student should be advised to secure a general education and that this general education should include courses in the major fields of study, then how much of the educational program should be devoted to science? Opinions concerning the answer to this question differ sharply. Some educators are inclined to believe that premedical students are getting too much science and that this scientific knowledge represents technical training rather than culture or education. Others are satisfied with the present state of affairs regarding the amount of science taken by premedical students but warn against a preponderance of science courses in the premedical program.

Fisher's (1950) statements are representative of the opinion that premedical students are getting too much science in their college education. He says:

> Medical educators and those preparing the young people of the country for medical schools are gradually becoming convinced that much of the present undergraduate training represents poor preparation for the study of medicine because it tends to overemphasize the natural sciences at the expense of the humanities and the social sciences.

How much is too much? Fisher (1950) studied the premedical programs of fifty-two liberal arts programs. He discovered that fifty-three per cent of the three-year programs and forty-seven

per cent of the four-year programs are devoted to the natural sciences. This is what he considers to be too much science. His conclusion is "that American colleges are permitting if not advocating an extremely high degree of undergraduate overspecialization."

Data to be presented in a later paper show that premedical students do take about half of their work in science. However, a committee on premedical education of the Ohio Academy of Sciences (1942) does not consider this a sign of scientific narrowness on the part of the premedical student. They say, "For a man to spend half of his college study, or even a little more than half, on the subjects fundamental to a profession as difficult and intricate as medicine seems to us scarcely unreasonable." This committee finds no significant problems in the present practices of premedical education.

Stetten (1946) argues for keeping the science courses to the minimum requirements of medical schools and devoting the major portion of the premedical curriculum to other studies. Although not ruling out specialization in science for the student with irresistible interest in science and with scientific ability, McGrath (1945) deems it desirable to advise premedical students in general to avoid early specialization in science. Rappleye (1940) also discourages making the college preparation for medicine largely science education. The University of Colorado School of Medicine (1949) and the University of Louisville School of Medicine (1949) warn applicants against taking a preponderance of science courses in their preparation for medical school. St. Louis University School of Medicine (1950) issues the statement:

> While in general a broad educational foundation is regarded as indispensable for determining the eligibility of applicants, it is obvious that the studies pursued during the college years must have some relationship to the subject matter of the medical curriculum.

The research dealing with the quality, quantity, and type of premedical work is reviewed by Stuit and Schlicher (1948). In summary they conclude:

> There is no evidence to show that a preponderance of science courses is an advantage in the study of medicine. One recent study points out that there is no significant relationship between the number of college hours of

instruction a student has completed in the natural sciences, social sciences, and humanities and success in medical school.

Gilmore (1936) declares flatly that students in science are trained but not educated. Meldrum and Cadbury (1946) oppose this when they claim the study of science has cultural as well as technical values. Nevertheless, they admit that the teaching of science often falls short of achieving the cultural objectives of scientific study.

The general opinion among medical educators regarding the amount of science in the premedical curriculum has two main points. To them it does not matter how much science a student takes so long as he includes the science necessary for entrance into medical school and does not take science courses to the exclusion of studies in the other major subject areas. Extremes in both directions are to be avoided.

Science in the Premedical Curriculum. The question to be considered now is just what is the premedical student expected to get out of his science courses. In the previous section it was seen that many medical educators object to the technical emphasis in college science courses. What function are the science courses to serve then?

Fisher (1950) says that science education must not strive for technical proficiency alone, but also for the eradication of prejudice, superstition, muddled thinking, and mental laziness. The motivation in the preliminary sciences is considered by Rappleye (1940) as probably more important than the amount of time and content of these subjects. Meldrum and Cadbury (1946) contend that the most valuable contribution of the study of science is the development of the ability to use the mind effectively. In addition they say that the most definite cultural values of the study of science are training in clear thinking and training in accurate observation.

Carmichael (1941) takes a viewpoint quite different from any of the above regarding the purpose of the sciences in the premedical curriculum. He feels that "Without a knowledge of the natural sciences, the scientific method and the scientific advances which have transformed modern society, it is impossible to comprehend the basis of American culture and progress." Keighton (1946) wants the science courses of the

premedical student to deal with fundamental principles and not with the application of the sciences to medicine. Calvin and Hendrix (1946) wish the sciences to furnish the basis for medical research.

There have appeared numerous articles concerning particular science courses in the premedical program. Physics has received the most attention because of the difficulties physics instructors have had in determining the content of a physics course for premedical students. It is advantageous to review these articles at this point.

1. *Chemistry.* Since general inorganic chemistry and organic chemistry are required by all medical schools, they do not get much treatment in the literature. It is the advanced courses that receive the most attention. Quantitative analysis is considered by Bergeim (1948) as a very important course for premedical students. He contends that medical schools have been able to do a better job of teaching biological chemistry since medical students have come prepared with a course in quantitative analysis. Bergeim believes that quantitative analysis is a discipline whose importance lies more in its part in the general education of the student than in the development of technique. The important point, according to Bergeim, is that the student has gained something of a quantitative idea which should persist throughout his later life. Baker (1946) has also studied the question of quantitative analysis for premedical students. The results of a questionnaire survey by him showed medical schools to be unanimously agreed on the desirability of quantitative analysis for premedical students although not all medical schools require this course.

Calvin and Hendrix (1946) emphasize the importance of the content of general inorganic, organic, and quantitative chemistry for the premedical student. With the medical sciences leaning so heavily on the basic sciences, Calvin and Hendrix (1946) want the premedical student well grounded in the fundamentals of chemistry underlying medicine. In addition to general inorganic and organic chemistry and quantitative analysis, Caldwell and Rogers (1946) advocate the inclusion of a course in physical chemistry as very valuable in the study of medicine.

2. *Biology*. A course in general biology is one of the minimum requirements set by the Council on Medical Education and Hospitals of the American Medical Association and the Association of American Medical Colleges. Hamburger (1945), acting as secretary for a group of biologists interested in teaching of general biology to premedical students, set down four conclusions reached by that group: (1) the course should always include laboratory work; (2) a separate course should be taught to premedical students; (3) inclusion of botany in the course should depend on local conditions; and (4) it is advantageous to have a course in chemistry precede the general biology course. A radical departure from the traditional college presentation of general biology is suggested by Fisher (1950). He wants the basic course in the biological sciences to be "truly a study of life."

Concerning advanced courses in biology, Hamburger (1945) reports a resolution in favor of requiring vertebrate embryology and comparative anatomy of all premedical students. Diehl (1939) studied the value of comparative anatomy as a preparation for the study of medicine by comparing the medical school grades of students with and without credits in this subject. He concludes that it is questionable whether the time and effort spent on comparative anatomy are justified. Kroatz (1939) made a rebuttal to Diehl's statements. He attacks Diehl's method of studying the question. Kroatz (1939) maintains that the value of comparative anatomy cannot be determined by studying the grades of students in medical school.

General physiology and genetics are mentioned by Hamburger (1945) as desirable advanced courses in biology. The advisability of including genetics in the premedical program provoked much discussion at the Conference on Premedical Education (1947). The consensus at this conference was that genetics, while beneficial to the premedical student, is not essential as a prerequisite for the study of medicine.

3. *Physics*. Discussions of physics for premedical students revolve around the question of how to teach physics to premedical students rather than whether or not physics belongs in the premedical curriculum. Physics is recognized as having more importance in modern medical science than formerly as a result

of developments in the application of physics to biology. Bluh (1949), Reed (1939), Setterfield (1948), Barkow (1938), and Harrington (1950) agree that the usual presentation of physics neglects biological application and that this situation should be remedied. A special course in premedical physics which uses illustrations from biology is outlined by Bluh (1949).

A questionnaire was used by Setterfield (1948) to study the problem of physics for premedical students. In response to the question, "What do you consider to be the most outstanding defect in premedical education in your schools?" seven of seventy-two colleges replying mentioned physics as the weak spot in their premedical programs. The principal criticism was the physics is too often taught as an engineering tool. Another question concerning means of improving the physics courses brought the general recommendation that more emphasis be placed on the understanding of physical principles and their application rather than on the mechanics of problem solving. Bluh (1949) agrees with this recommendation.

Cultural aims in physics are indicated by Bluh (1949). He claims that humanistic values are implicit in any science course and can well be put in relief. Regarding physics in particular, Bluh considers illustrations from biology as "humanizing elements" desirable for inclusion.

The advisability of advanced courses in physics for premedical students is investigated by Setterfield (1948) in his questionnaire. From his data he concludes that most medical and premedical educators do not feel that a special course in physics dealing with instruments, their operation and application, is advisable. Bluh's ideas are in accord with this, but he feels an advanced course in physics is desirable when the student has reached a greater maturity to appreciate the benefits of such a course. In view of the increased importance of physics in medicine, the University of Pennsylvania School of Medicine (1949) recommends advanced physics courses for students with an interest and aptitude in this field.

4. *Mathematics.* Mathematics in most colleges is a prerequisite for the physics course. Besides its utility in the study of physics, other advantages have been ascribed to mathematics.

Zapffe (1940a) is of the opinion that mathematics is a cultural subject because "it is splendid mental gymnastics." Essentially the same opinion is held by Skiles (1942). The application of mathematics to medical science is stressed by the Johns Hopkins University School of Medicine (1949) in its recommendation to applicants which states:

> In view of the increasing employment of mathematical concepts in the medical sciences, students expecting to take up the study of medicine are advised to include one year of mathematics, calculus if possible, in their college course.

Nonscience Courses in the Premedical Curriculum. English is the only nonscience course among the requirements set up by the Council on Medical Education and Hospitals for admission to approved medical schools. Other nonscience courses required by particular medical colleges include psychology, history, government, foreign language, and unspecified social science courses. An analysis of the recommendations of American medical colleges shows that the nonscience courses or study areas most highly recommended are social sciences, psychology, history, economics, and political science. With the exception of English, there is less agreement among medical and premedical educators on the values of particular nonscience courses than on the values of particular science courses. The comments of these men and the statements of medical schools on English, foreign language, social science, psychology, and philosophy are given below.

1. *English.* Most of the medical schools adhere closely to the minimum of six semester hours set by the Council on Medical Education and Hospitals and the Association of American Medical Colleges. Two medical educators suggest that perhaps this minimum does not satisfy the needs of premedical students. Ivy (1948) believes that too many students lack competence in written communication. Zapffe (1940a) favors more stress on English in the preparation of students for medical studies. The teaching of English should be the concern of all college teachers, according to Keighton (1946), because the teaching of the art of communication is one of the aims of the liberal arts college. The Medical College of Virginia (1950)

expects its students to have a thorough training in the fundamentals of English so that they are capable of expressing themselves logically, clearly, and concisely. The statement of Indiana University School of Medicine (1949) relative to English is worth quoting here:

> . . . it is recommended that the premedical student prepare himself in the arts of communication so that he may write and speak well. The courses in composition and speech are designed to fill a need which most practicing physicians have indicated is one of the most important in their experience. In the field of speech, courses in public speaking and debating will contribute skill in logical analysis and ability to speak convincingly before audiences.

Besides the elements of grammar and composition, literature is considered important in the education of the premedical student. Reinke (1936) feels that an appreciation of literature is an essential characteristic of an educated man. The University of Cincinnati College of Medicine (1949) emphasizes the importance of familiarity with outstanding works in English literature. In order here perhaps is a statement of Conant (1939) concerning the narrowness of physicians:

> If doctors could be made to acquire the habit of reading something other than scientific or professional literature on the one hand, or trash on the other, much of the complaint about the narrowly trained specialist would disappear.

2. *Foreign Language.* The opinions regarding foreign language for premedical students vary widely. This is probably because of differences regarding the objectives to be achieved in a study of foreign language. These differences are illustrated well by the response to a questionnaire study by Alpha Epsilon Delta (1947) of the question asked to both medical and premedical educators. The question read: "What are your views regarding the requirements of a reading knowledge of a foreign language for admission to medicine?" The general opinions expressed by the results of this questionnaire are that language study is not necessary for medicine and should not be required. However, a majority of the replies indicated the cultural value of foreign language study as being more important than its utilitarian value. Only a very few medical educators feel that the amount of foreign language studied in two years of college

gives the student sufficient command of a language to enable him to use it. For cultural purposes, most of the educators feel that it makes little difference what language is studied. In its evaluation of the answer to its questionnaire, Alpha Epsilon Delta (1947) makes a very interesting prediction:

> We venture to predict that as more of the present generation of medical students become the teachers of the next that the use and study of foreign languages, especially modern languages, will become still less emphasized.

Carmichael (1941) emphasizes both the cultural and the utilitarian objective of foreign language study. In commenting on the value of studying a foreign language he says, "There is perhaps no subject so valuable as a means of developing accuracy of expression and hence accuracy of thought as this study." In a discussion at the 1947 Alpha Epsilon Delta Conference on Premedical Education, Tucker expressed his hope that medical schools will drop the foreign language requirement entirely since medical students could not use the language they had studied.

The study of classical languages is favored by Skiles (1942), Zapffe (1940a), The State University of Iowa College of Medicine (1950), and a number of those answering the Alpha Epsilon Delta questionnaire (1947). All of these consider Greek and Latin as cultural subjects and valuable in understanding medical terminology.

3. *Social Science.* Although not widely required by medical schools for admission, the social studies are the most highly recommended studies for premedical students. In a previous section of this paper, it was observed that several medical educators considered one of the objectives of premedical education to be concerned with social consciousness, understanding the world and human nature, appreciation of social responsibilities and other similar expressions. Skiles (1942) quotes Day as saying that the future doctors need social training in the social sciences.

The research work of the Cornell University Medical College (1949) on the effects of sociological and psychological factors upon sickness and health indicate a new trend in medi-

cine. Concerning the objectives of social science in the premedical curriculum, Fisher (1950) says:

> The social sciences must aid our youth by suggesting feasible social predictions, consistent with an era in which distances will cease to be a barrier separating different cultures.

History is the only particular social science course that is treated in the literature. Keighton (1946) includes history as one of the studies essential to a general education. Carmichael's (1941) lengthy comment on history is well worth quoting:

> History is more than a study of the past as bequeathed to the present. It develops a sense of perspective that applies in the consideration of any problem. . . . Nothing is viewed as an end of itself alone. Any given fact is recognized as part of a pattern, and the natural inclination of the history student is to comprehend the pattern.

Zapffe (1940) opposes history in the premedical curriculum. He feels that the student has had enough history by the time he reaches college.

Three medical schools are quite explicit in encouraging applicants to study the social sciences. Southwestern Medical School of The University of Texas (1950) urges the prospective medical student to have as broad a concept of human relations as possible. The University of Chicago School of Medicine (1950) desires its applicants to have an understanding of the organization of society and of its machinery and history. The social obligations of the physician are considered by the University of Colorado School of Medicine (1949) as necessitating courses enabling the student to understand these obligations.

4. *Psychology.* Among his recommendations to premedical students, Zapffe (1940a) includes a course in psychology. Middleton (1940) discovered this recommendation to be fairly general among medical schools while making a study of the attitude of medical schools toward the undergraduate course in abnormal psychology for premedical students. While favoring a course in general psychology, most of the medical schools Middleton studied were either indifferent or opposed to an undergraduate course in abnormal psychology. Levir (1939) believes a foundation in psychology will give the physician a

helpful approach to the patient. Opposing psychology in the premedical curriculum is Kennedy (1939) who claims it gives "a distorted view of life."

5. *Philosophy*. Philosophy is placed by both Skiles (1942) and Zapffe (1940a) with mathematics and the classics as being outstanding as contributing to the cultural education of the premedical student. Zapffe feels that philosophy gives a student good judgment and the ability to reason and think logically. Setterfield (1948) agrees that philosophy has cultural value. Because medical students fail to see the applications of philosophy to medicine and to life in general, the University of Georgia Medical School (Mettler, 1940) arranges a lecture on "Philosophy in Daily Life" as part of its orientation program for entering students. Fisher (1950) has this to say about philosophy:

> In the ideal situation, philosophy offers to the student the best thought and hypotheses formulated by the wise men of all times. The nature of causality, free will and truth are problems which enter into the thoughts of every thinking person.

Leake (1952) emphasizes the importance of philosophy, especially logic, aesthetics, and ethics, to the general practitioner. He feels that logic has its value in the development of a body of scientific knowledge and that the application of this knowledge to a purpose involves aesthetics. Ethics assists in proper evaluation of purposes.

Summary

Much has been written by medical educators regarding the various problems of premedical education. In reviewing the literature on the problem of what subjects the premedical student should study in college, attention was first given to the objectives of premedical education. Medical educators seem agreed that "premedical education" does not denote a type of education different from the education of other students but refers merely to the education of premedical students. The dissatisfaction of medical educators with the vocational implications of the term "premedical education" gives a clue to their general ideas regarding the objectives of the education of

premedical students. The common opinion is that the premedical curriculum should afford the student opportunities to develop those traits and characteristics usually regarded as qualities of a cultured or educated man and at the same time include enough science to enable him to undertake medical studies. Among these cultural objectives medical educators include such things as training of the mind, development of the will, appreciation of beauty, emotional adjustment and maturity, social consciousness, acquisition of good work habits, and development of the scientific attitude. The consensus is that the objectives of the education of premedical students are generally not different from the objectives of the education of other students.

General education has been proposed by a number of medical educators as suitable to meet the objectives of premedical education. By general education is meant study in all the major fields of knowledge to give the student a "well-rounded" educational experience. Scientific studies belong in this general education, but not to the exclusion of studies in the other major branches of knowledge. It is doubtful that science should be limited to the minimum requirements of medical schools.

The science education of the premedical student should prepare him for medical studies and develop the scientific attitude in him. In addition to the inorganic and organic chemistry already required by medical schools, medical educators recommend quantitative analysis and physical chemistry as valuable for medical studies. Biology subjects considered worthy of being included in the premedical curriculum are general biology, vertebrate embryology and comparative anatomy. The physics course for premedical students should include illustrations from biology and avoid too many engineering examples and problems. Premedical students are urged to take at least a year of mathematics.

Although most of the American medical schools adhere closely to the minimum requirement of one year of English, medical educators feel that the importance of English to the physician demands more attention. Not only are the elements of grammar and composition considered important, but also English literature.

Opinions on the value of foreign language to the premedical student vary. The general opinion of medical educators is that language study is not necessary for medicine and should not be required. However, the majority of these men recognize the cultural value of foreign language study and recommend foreign languages for this value. It makes no difference what language is studied. Greek and Latin may also be helpful in understanding medical terminology.

The study of the social sciences is advocated by medical educators because of its importance in helping the student understand society and human nature. The only social science singled out for comment is history which is generally recommended.

It is not surprising to find psychology receiving the approval of medical educators for inclusion in the premedical program since it stands so high among courses recommended by medical schools. Philosophy has also been proposed by several as a valuable cultural course.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

Hopeful Holidays, 1952

Serious Holiday Reading: C Ailen probes in *Ushant: An Autobiographical Narrative* (Little Brown, Boston, 52, 328pp. $4.5). F Barlow describes *Mental Prodigies* (Philosophical Library, NY, 52, 256pp, $4.75). I Berenblum portrays *Man Against Cancer* (Johns Hopkins Univ Press, Baltimore, 52, 223pp, $3). AH Brodrick climbs *The Tree of Human History* (Philosophical Library, NY, 52, 253pp, $4.75). What B Cerf is *Good for a Laugh* (Hanover, NY. 157pp, 52, $2). KMT Chrimes offers example of *Ancient Sparta* (Philosophical Library, NY, 52, 542pp, $8.75). JC Clark & MT Halbouty recount thrill of Texian *Spindletop* (Random House, NY, 52, 312pp, $3.95). JGD Clark surveys *Prehistoric Europe: The Economic Basis* (Philosophical Library, NY, 52, 368pp, $12). R & J Debos review *The White Plaque* (Little Brown, Boston, 52, 284pp, $4). Texian CG Hartman writes fully & well on *Possums* (Univ Texas Press, Austin, 52, 174pp, $6). WV Houston & Co offer *The Scientists Look at Our World* (Univ Pennsylvania Press, Phila, 52, 147pp, $3). Our *Old Egyptian Medical Papyri* at last appears (Univ Kansas Press, Lawrence, 52, 113pp, $2). R Livingstone relates *Education & the Spirit of the Age* (Oxford Univ Press, London, 121pp, 52, $2). P Locatelli of Pavia eulogizes his townsman Camillo Golgi (1843–1926) (*Sci Med Ital* 2/355/52). NRF Maier states *Principles of Human Relations* (Wiley, NY, 52, 483pp. $6). Our P Romanell analyses the *Making of the Mexican Mind* (Univ Nebraska Press, Lincoln, 52, 223pp, $3.75). W Saroyan becomes *The Bicycle Rider in Beverly Hills* (Scribner's NY, 52, 178pp, $3). WV Silverberg discusses *Childhood Experience & Personal Destiny* (Springer, NY, 52, 298pp, $4.5). HW Spiegel edits *Development of Economic Thought*, a timely topic (Wiley, NY, 52, 823pp $6.5). DA Talbot describes (remember?) *Contemporary Ethiopia* (Philosophical Library, NY, 52, 277pp, $4.5). S Tax edits *Indian Tribes of Aboriginal America* (Univ Chicago Press, 52, 420pp, $7.5). V du Vigneaud looks back on *A Trail of Research* (Cornell Univ Press, Ithaca, NY, 52, 204pp, $3.25). CS White & OO Benson Jr well edit *Physics & Medicine of the Upper Atmosphere* (Univ New Mexico Press, Albuquerque, 52, 635pp, $10).

Notable Reprints: C Darwin *Journal of Researches* (Hafner, NY, 52, 629pp, $7.5); S Freud *The Case of Dora & Other Papers* (Norton, NY, 52, 243pp, $3.5); F Galton *Hereditary Genius: An Inquiry into its Laws & Consequences* (Horizon, NY, 52, 406pp, $4).

Current & Basic: VT Bowen edits *The Chemistry & Physiology of the Nucleus* (*Exp Cell Res*, Suppl 2, Academic Press, NY, 52, 402pp). A Butenandt describes action of hereditary factors (*Endeavor* 11/188/52). CA Handley & JH Moyer analyse mechanism of renal effects of morphine (*Arch Internat Pharmaco* 90/185/52). Our TC Hsu well shows characters

of human chromosomes (*J Heredity* 43/167/52). B Josephson & G Dahlberg analyse blood cell & chemistry variations with age, sex & season (*Scand J Clin Lab Invest* 4/216/52). AS Losner describes the ballistoscope for study of cardiac impulse (*Proc Soc Exp Biol Med* 81/1/52). EG McQueen & AJ Palmer find hexamethonium bromide helpful in hypertension (*Med J Austral* 2/425, 428 /Sept 27/52). CW Mushett & Co show hydroxo-cobalamine counteracts cyanide toxicity (*Proc Soc Exp Biol Med* 81/234/52). WDM Paton & EJ Zaimia review the methonium compounds (*Pharmacol Rev* 4/219/52). M Pickford dittos antidiuretic substances (*ibid* p 254). KL Pines & Co show formation of cyanide from thiocyanate due to red cell enzyme (*Proc Soc Exp Biol Med* 81/149, 160/52). Our M Pollard & Co give infra red absorption spectra of viruses (*Ibid* p 10). M Richards reviews iodine metabolism (*Schw Med Wchnschr* 82/1073/Oct 18/52). So does DS Riggs (*Pharmacol Rev* 4/284/52). AS Wiener offers history & bibliography of rhesus blood types (*J Hist Med* 7/369/52).

NOTE: If you're interested in attractive holiday books, the nicest and neatest in titles & typography are those issued by the Peter Pauper Press of Mt. Vernon, NY.

JANUARY, 1953

GENERAL: Allergy pioneers P Portier, C Richet, H Dale celebrate semicentenary of discovery of anaphylaxis (*Acta Allerg* 5/173–213/52). BJ Anson pictures influence of Vesalian *Fabrica* of 1543 (*Anat Rec* 114/295–331/52). HJ Burrows shows influence of posture notes of Leonardo da Vinci (1452–1519) on N Andry (1658–1742) founder of orthopedics (*J Bone Jt Surg* 34/541–544/52). J Dobson gives account of Clopton Havers (1657–1702) pioneer of osteogeny (*ibid* p 702). P Diepgen revives Sudhoff's *Arch Gesch Med*. JG Gilbert tries *Understanding Old Age* (Ronald Press, NY, 52, 422pp, $5). DR Griffin & E Mayr analyse bird migration (*Biol Rev* 27/359/52). P Mullahy edits The Contributions of Harry Starck Sullivan (1893–1950), symposium on Interpersonal Theory (Heritage, NY, 52, 238pp, $3.5). DH Robertson considers economics in *Utility & All That* (Allen & Unwin, London, 52, 320pp, 18s). EH Sellards gives facts on *Early Man in America* (Univ Texas Press, Austin, 52, 211pp, $4.5). C Singer translates & annotates Galen's elementary course on bones (*Proc Roy Soc Med* 45/767/52).

CLINICAL: E Castillo & J Esplugues recommend papaverine in cardiovascular therapy (*Med Espan* 28/91/52). M Demole discusses therapeutic problems of obesity (*Schw Med Wchnschr* 82/1952/Nov 29/52). W Dock edits *Disorders of the Circulatory System* (Macmillan, NY, 304pp, $5.5). N Dorken finds high incidence of constrictive pericarditis due to Tb (*Bull Soc Turq Med* 18/269/52). JM Essenberg experimentally causes primary lung cancer by cigarette smoke (*Science* 116/561/Nov 21/52). EE Fischel & Co review treatment of rheumatic fever (*Med* 31/331/52). M Foldi & Co discuss lymph circulation in pathogenesis of edema (*Acta Med Acad Sci Hung* 3/259/52). AL Gropper & Co review plasma expanders & offer full bibliography (*Surg Gyn Obs* 95/521/52). RA Hingson & FHJ Figge re-

port development of jet injection in parenteral therapy (*Cur Res Anesth Analg* 31/361/52). E Jalovisto & S Pika find increased redox potential with increased reticulocytes (*Ann Med Exp Biol Fenn* 30/361/52). SM Jordan's committee on surgical procedures in gastric ulcers reports inconclusively (*Gastroenterol* 22/295/52). D Muting finds high methionine excretion in liver disorder & low in nephrosis (*Ztschr klin Med* 150/1/52). JE Richardson describes pancreatic islet cell tumors causing cerebral disease (*Lancet* 2/1054/Nov 29/52). E Rothlin & R Bircher discuss allergy, the autonomic nervous system & ergot alkaloids (*Prog. in Allerg* 3/434/52). IH Scheinberg & D Gitlin note deficiency of ceruloplasmin in hepatolenticular degeneration (*Science* 116/484/Oct 31/52). HJ Steinke & G Vogel find curare diabetes due to adrenal response to hypoxia (*Pfluger's Arch ges Physiol* (256/952). P Story describes testing skin disinfectants with 1% iodine in 70% alc killing all test organisms in 30 sec (*Brit Med J* 2/1128/Nov 22/52). CB Thomas suggests hypertension as effect of recessive gene, with expression as coronary disease in males & hypertension in females (*Am J Med Sci* 224/367/52). A Turchetti & G Schirosa describe evolution of pulmonary hypertension (*Cardiol* 21/129/52).

Basical: RL Beard & KF Heumann describe chemical-biological coordination center (*Science* 116/553/Nov 21/52). O Bodansky edits 2nd ed *Biochemistry of Disease* (Macmillan, NY, 52, 1208pp, $12). AC Corcoran edits symposium on methods of renal study (*Methods in Medical Research* 5/134–267/52, Yearbook Publ, Chicago). H Croxatto & Co find renin increases diuresis (*Science* 116/507/Nov 7/52). CND Cruickshank & EJL Lowbury appraise possible local application toxicity of antibiotics by tissue culture (*Brit Med J* 2/1070/Nov 15/52). N Ercoli & GM Carminati note anti-spirochetal interference between antibiotics and arsenoxide (*Science* 116/579/Nov 28/52). WC Grant & WS Root review stimuli for erythropoiesis (*Physiol Rev* 32/449/52). FA & P Holton find vasodilator substance in spinal root extracts (*J Physiol* 118/310/52). DA McDonald finds systolic aortic flow is 60cm/sec, with backflow to 25cm/sec at diastole, with turbulence (*ibid* p 328, 340). J Macleod edits symposium on biology of the testes (*Ann NY Acad Sci* 55/543–742/52). H Maske & Co find alloxan gives short rise in serum & urine zinc (*Arch Exp Path Pharmakol* 216/457/52). JH Milstone thinks better agreement is possible on theories of blood coagulation (*Med* 31/411/52). Note Symposium on method of separation of complex mixtures (*Methods in Medical Research* 5/1–133/52, Yearbook Publ Chicago). S Zlatarov & M Hallo indicate thallium inhibits alkaline phosphatase (*Acta Med Acad Sci Hung* 3/331/52).

February, 1953

Laboratory: EH Bourne & JF Danielle edit *International Review of Cytology* (Academic Press, NY, 52, Vol I, 380pp, $7.8). . . . MAB Brazier discusses *The Electrical Activity of the Nervous System* (Macmillan, NY, 53, 220pp, $5). . . . H Druckrye considers biochemorphology of carcinogens (*Arzneiforsch* 2/503/52). MD Eaton proposes local inhibition of viral growth by interfering with high energy phosphate synthesis by diamidines

or dinitrophenol (*Arch ges Virusforsch* 5/53/52). . . . L Engel cautions on medical statistics (*Harper's*, Jan/53, p 77). . . . .US von Euler concludes noradrenalin maintains circulatory homeostasis while adrenalin is secreted in response to stress (*Scand J Clin Lab Invest* 4/254/52). . . . L Festinger & Co discuss mechanism of group influence when extreme deviation occurs (*Human Rel* 5/327/52). . . . RW Holley describes steric inhibition of amide resonance in enzyme action (*Science* 117/23/Jan 9/53). . . . PL Kirk offers *Crime Investigation: Physical Evidence & the Police Laboratory* (Interscience, NY, 53, 806pp, $10). . . . WD McElroy & B Glass edit *Phosphorus Metabolism II* (Johns Hopkins Press, Balt, 53, 965pp, $11). . . . AM Pappenheimer edits *The Nature & Significance of the Antibody Response* (Columbia Univ Press, NY, 53, 270pp, $5). . . . H Pieron analyses *The Sensations: Their Functions, Processes & Mechanisms* (Yale Univ Press, New Haven, 53, 310pp, $6). . . . N Porges describes snake venom biochemistry & action (*Science* 117/44/Jan 16/53). . . . HE Rawlinson & GB Pierce show mammary glands store & secrete iron (*ibid* p33/Jan 9/53). . . . AC Redfield edits *Marine Fouling & Its Prevention* (US Naval Inst, Annapolis, Md, 398pp, 52, $10). . . . A Sarachek & GF Townsend find mitochondrial disruption by ultraviolet radiation (*Science* 117/31/Jan 9/53). . . . H Saurano studies histopathology & function of senile ovary (*Ann Chir Gyn Fenn*, Suppl 1, Vol 41, Helsinki, 52, 66pp). . . . Our GR Seaman describes new type phosphagen in protozoa (*Biochim Biophys Acta* 9/693/52). . . . T Sjostrand supports notion of lesser circulation as effective blood reservoir (*Acta Physiol Scand* 26/312/52). . . . HA Teitelbaum discusses rhythmic activity of nervous system (*Phil Sci* 20/42/53). . . . PE Voorhoeve defines Weber factor as fraction of standard stimulus giving 50% change in sensation (*Acta Physiol Pharmacol Neerl* 2/516/52). . . . H Weinfeld & AA Christman show methyl uric acids in urine after caffeine & theophylline (*J Biol Chem* 200/345/53).

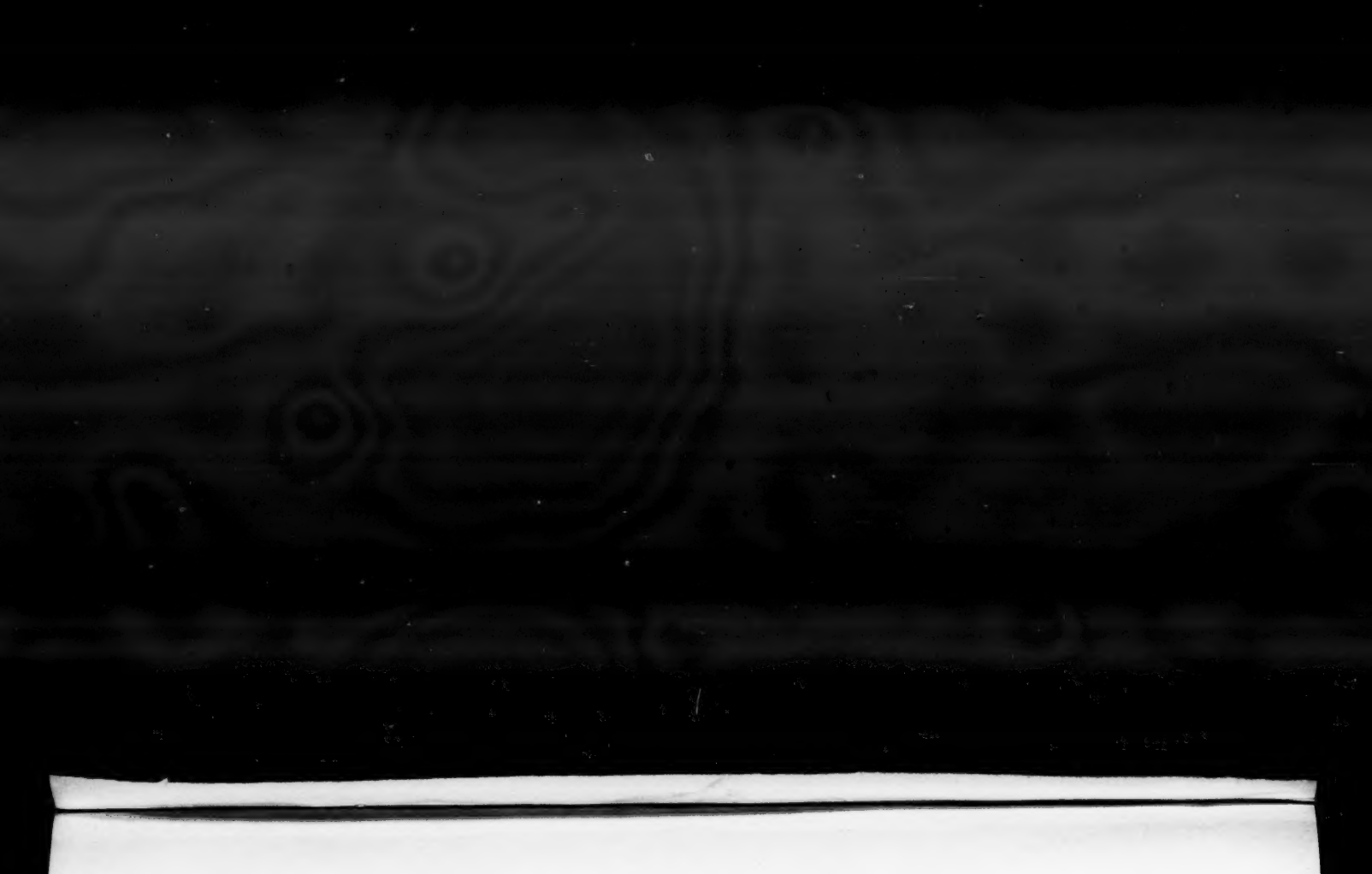
Clinic: BS Abeshouse discusses differential diagnosis of pancreatic & renal cysts (*Surg Gyn Obs Internat Abst Surg* 96/1/53). . . . EC Albritton offers helpful *Standard Values in Blood* (WB Saunders, Philadelphia, 53, 199pp, $4.5). . . . HR Bierman reviews chemical agents in neoplastic disease (*Calif Med* 78/44/53). . . . R Biggs & Co describe "Christmas Disease" (named for patient "Christmas") similar to hemophilia (*Brit Med J* 2/1378/Dec 27/52). . . . J Delay & JE Thuillier curarize by rectal suppository (*Science* 117/57/Jan 16/53). . . . KJ Franklin discusses uterine influences on intrarenal blood distribution (*Lancet* 2/1141/Dec 13/52). . . . AR Frish & Co report new safe & efficient mercurial diuretic, succinylcarbomethylmercaptomercuri propyl carbamide, "Diurgin" (*Acta Med Scand* 144/85/52). . . MR Harrower pleasantly dialogues *Appraising Personality: The Use of Psychological Tests in the Practice of Medicine* (WW Norton, NY, 53, 216pp, $3.75). . . . I Innerfield & Co suggest intravenous trypsin as safe agent for thrombolysis & anticoagulating effect (*J Clin Invest* 31/1049/52). . . . P Kielholz discusses management & prognosis of chronic morphinism (*Schw Med Wchnschr* 82/1325/Dec 27/52). . . . JS Levy & RW Talley report mercapto-arsenal effective & safe in amebiasis (*Gastro-*

enterol 22/588/52). . . . WS McCann explains W Harvey's (1578–1657) 2 kinds of death in failing circulation from lack of excess, & emphasizes importance of CO_2 as hemodynamic factor (*Calif Med* 78/1/53). . . . DW Richards analyses cardiac & pulmonary dyspnoea (*Circula* 7/15/53). . . . MI Sturnick & SL Gargill enthuse over safe estrogenic effect of methoxy allenolic acid, "Vallestril" (*New Eng J Med* 247/829/Nov 27/52). . . . P Theron & Co say venous pressure is index of oligemia (*S Afr J Med Sci* 17/53/52). . . . JW Todd discusses precision in tentative explanation of patient's symptoms or diagnosis (*Lancet* 2/1235/Dec 27/52). . . . RW Williams & WE Judson (*New Eng J Med* 248/48/Jan 8/53) confirm B Bhatia (*J Indian Med Asso* 11/262/42) that oral rauwofia serpentina ("Serpina") is helpful well tolerated vascular relaxant in hypertensive patients.

MARCH, 1953

GENERAL: Sir Russell Bain analyses neurology of last illness of cranky John Hunter (1728–1893) (*Brit Med J* 2/1371/Dec 27/52). . . . Existentialist S. de Beauvoir's *The Second Sex* is translated & edited by HM Patshley (Knopf, NY, 53, 732pp, $10) & well reviewed by K Menninger, P Wylie, A Montagu & Co (*Sat Rev*, Feb 21/53. . . . JF Fulton eulogized Lewis Hill Weed (1886–1952) (*Yale J. Biol Med* 25/215/52). . . . GB Gruber reviews century of *Munchener Medizinische Wochenschrift* founded by Hermann Oettinger (1802–1855) & Alois Martin (1818–1891) (*Munch Med Wchnschr* 95/Jan 2/53). . . . WR Hess discusses physiologic basis of aesthetics (*Helv Physiol Acta* 10/462/52). . . . Essays of W DeB MacNider (1881–1952) are collected in *The Good Doctor & Other Writings* (Univ No. Carolina Press, Chapel Hill, 53, 224pp, $5). . . . A von Muralt & D Nachmansohn offer biobibliography of Otto Meyerhoff (1884–1951) (*Ergeb Physiol* 47/1/52). . . . R Waters offers fine appreciation of AE Guedel, great anesthetist (*Brit J Anesth* 24/292/52).

BASIC: BM Graganca & JH Quastel study enzyme inhibitions by snake venoms (*Biochem J* 53/88/53). . . . H Croxatto & Co suggest renin diuresis is related to available NaCl (*Acta Physiol Latino-Amer* 2/159/52). . . . W Gooddy & M Reinhold consider human orientation in space (*Brain* 75/472/52). . . . GS Gordan & Co show brain oxidizes more than glucose & that steroids alter brain metabolism (*Calif Med* 78/87/53). . . . D Grob & AM Harvey discuss effects & treatment of nerve gas poisoning (*Am J Med* 14/52/53). . . . C Heymans & Co. review pressoceptors & blood pressure regulation (*Circul Res* 1/3/53). . . . WM Holt & S Warren describe freeze-drying tissues for autoradiographic study (*Lab Invest* 2/1/53). . . . HA Itano describes human hemoglobin (*Science* 117/89/Jan 30/53). . . . PA Khairallah & WFHM Mommaerts find mammalian myocardium contains 5×10^{-6}mol nucleotide (2.5 mgm/gm) as ATP, diminished in systole to nucleotide H (*Circul Res* 1/8, 12/53). . . . AI Lansing & Co show rope-like fibrils of elastic tissue has amino-acid composition (*Anat Rec* 114/555/52). . . . MC Nath & CH Chakrabarti find acetoacetate & hydroxy butyrate may cause riboflavin & nicotinic acid deficiency (*Proc Soc*

Exp Biol Med 82/5/53). . . . BA Pethica & JH Schalman discuss physical chemistry of hemolysis by surface-active agents (***Biochem J*** 53/177/53). . . . CL Pirani & SM Levenson find ascorbic acid necessary for maintenance of wound healing (*Proc Soc Exp Biol Med* 82/95/53). . . . E Pollard says primary ionization releases energy by incident particles on large molecular aggragates with loss of activity giving clue to size of such molecules (*Am Sci* 39/99/53). . . . Our RH Rigdon & HG Swann find clinical results of anoxia due to cerebral injury not adrenal insufficiency (***Proc Soc Exp Biol Med*** 82/111/53). . . . JF Rinehart & Co show importance of muco-carbohydrate-protein in flomerular pathology (*Am J Path* 29/21/53). . . . A Rosenblueth reviews local responses of axones (***Ergeb Physiol*** 47/24/52). . . . R Setlow & P Doyle say inactivation of insulin by fast charged particles shows insulin unit of activity is spherical with molecular wt 23,000 (*Arch Biochem Biophys* 42/83/52). . . . M Stefanini reviews mechanism of blood coagulation (*Am J Med* 14/64/53). . . . GW Thorn & Co discuss adrenocortical steroids & ACTH (*New Eng J Med* 248/232/Feb 5/53). DW Woolley gives evidence for synthesis of B_{12} by spontaneous tumors (*Proc Nat Acad Sci* 39/6/53).

CLINICAL: H Arnoldsson & W Pipkorn discuss allergy treatment with ACTH (*Schw Med Wchnschr* 89/10/Jan 3/53). . . . HW Brown edits symposium on antibiotics in tropical diseases (*Ann NY Acad Sci* 55/967–1284/Dec 30/52). . . . J Gerrard reviews kernicterus (*Brain* 75/526/53). . . . H Kalliala & Co note antidiuresis & increased Na excretion during nursing (*Ann Med Exp Biol Fenn* 30/96/52). . . . LP LeQuesne & AAG Lewis indicate post-operative water & sodium retention due to stress factors (*Lancet* 1/153/Jan 24/53). JH McNinch edits symposium on epidemic hemorrhagic fever (*Ann Int Med* 38/53–112/53). . . . Our JL Otton proposes bettering drug addiction control (*Texas S J Med* 49/56/53). . . . FP Shidler & EF Holman discuss mediastinal tumors (*Stanford Med Bull* 10/217/52). . . . WF Von Oettingen offers *Poisoning: A Guide for the Clinician* (Hoeber, NY, 53, 534pp, $10). . . . S Waldman & L Pelner find pyrodoxine breaks mercurial fastness in patients with congestive heart failure (*Am J Med Sci* 225/39/53). . . . HB Weinberg urges prevention of iatrogenic ("caused by doc") heart disease (*Ann Int Med* 38/9/53). . . . GEW Wolstenholme & MP Cameron edit fine *Ciba Foundation Colloquia on Endocrinology* (Blakiston, Phila., 52, 4 vols, 315, 429, 380, 591pp, $20), covering steroids & anterior pituitary.